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ASSESSING THE ENVIRONMENTAL RISKS ASSOCIATED WITH CONTAMINATED SITES

Elena Boriani

Thesis submitted for the degree of

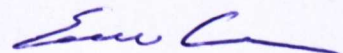
Doctor of Philosophy

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**Mario Negri Institute for
Pharmacological Research**



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To Life,

**“Ogni vita, la mia e quella di un albero,
è parte di un tutto dalle mille forme che è *la vita*”.**

Tiziano Terzani, Un altro giro di giostra.

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ABSTRACT

A risk assessment strategy considering the impact of chemicals on the whole ecosystem has been developed in order to create a sound and useful method for quantifying and comparing global risks posed by the main different hazardous chemicals found in the environment. This index, called the Environmental Risk Index for a Complete Assessment (ERICA), merges in a single number the environmental assessment, the human health risk assessment and the uncertainty caused by missing or unreliable data. ERICA uses a scoring system with parameters for the main characteristics of the pollutants. The main advantage is that it preserves a simple approach by condensing in this single value an analysis of the risk for the area under observation.

The availability and reliability of the data is an important part of the work done to build the index. Experimental and predictive data were compared to evaluate the reliability. Data were derived both from literature sources (experimental models mainly) and predictive models. ERICA can be considered a diagnostic and prognostic tool for environmental contaminants in critical and potentially dangerous sites, such as incinerators, landfills and industrial areas or in broader geographical areas. The application of the proposed integrated index provides a preliminary quantitative analysis of possible environmental alerts due to the presence of one or more pollutants in the investigated site.

This thesis presents the method and the equations behind the index and a first case study based on the Italian legislation and a pilot study on a location on the Italian seacoast.

Keywords: risk index, pollutants, ecotoxicity, human risk assessment, environment risk assessment, integrated strategy, predictive methods, uncertainty.

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Lombardo, A. Roncaglioni, **E. Boriani**, C. Milan, E. Benfenati. "Assessment and validation of the CAESAR predictive model for Bioconcentration factor (BCF) in fish". Chemistry Central Journal 2010, 4(Suppl 1):S1 (29 July 2010).

E. Boriani, A. Mariani, D. Baderna, C. Moretti, E. Benfenati. "ERICA: a multiparametric toxicological risk index for the assessment of environmental healthiness." Environmental International, 36 (2010) 665–674

V. Senese, **E. Boriani**, A. Mariani, D. Baderna, M. Lodi, S. Testa, A. Finizio, E. Benfenati. "Definition of an ecotoxicological classification risk index for soil (ECRIS), an Italian landfill case study". *Chemosphere, Volume 80, Issue 1, June 2010*

C. Zhao, **E. Boriani**, A. Chana, A. Roncaglioni, E. Benfenati. "A New Hybrid QSAR Model for Predicting Bioconcentration Factor (BCF)". *Chemosphere*, 73, 1701-1707, 2008.

C. Porcelli, **E. Boriani**, A. Roncaglioni, A. Chana, E. Benfenati "Regulatory perspectives in the use and validation of QSAR. A case study: DEMETRA model for daphnia toxicity" Environ Sci Techno 2008 ; 42: 491-496.

List of Acronyms / Abbreviations

AE = Average Exceeding

AQI = Air Quality Index for air macropollutants

BCF = Bioconcentration Factor

BMF = Bio Magnification Factor

CSA = Chemical Safety Assessment

EF = Environmental Fate

EFI = Environmental Fate Index

EQ = Ecotox Quality Index

EQI = Ecotoxicological Quality Index

ER = Exceeding Risk

ERI = Environmental Risk Index

ERICA = Environmental Risk Index for Coupled assessment

ERIE = Environmental Risk Index and Exceeding value

HC = Cancer Risk Index

HCR = Human Cancer Risk

HQ = Human Quality Index

HTI = Human Toxicological Index

ITE = Integrated Threshold Exceeding

LOAEL = Lower Observed Adverse Effect Level

Kow = octanol–water partition coefficient

Koc = soil adsorption coefficient

ME = Maximum Exceeding

NAAQS = National Ambient Air Quality Standard

NEP = Number of Pollutants Exceeding risk threshold

NIC = Number of Investigated Priority Compounds

NOEL = No Observed Effect Level

PBT Persistent Bioaccumulative Toxic

PEC = Predicted Environmental Concentration

PI = Pollutant Risk Index

PNEC = Predicted No-Effect Concentration

RT = Risk Threshold

SRI = Substance Risk Index

SSV = Soil Screening Value

BAF = Bio Accumulator Factor

BCF = Bio Concentration Factor

D = environmental Distribution

SF = Slope Factor

SMILES = Simplified Molecular Input Line Entry Specification

RfD = Reference Dose

INTRODUCTION

Many risk indicators, priority systems and schemes to screen chemicals for potential adverse effects once released into the environment have been published. Several indicators have been applied in various countries, with different goals and methods.

A number of research organizations have started exploring the state of the art of risk indicators, in particular for pesticides, examining the outcome and limitations of different approaches with the goal to harmonize the use of these indicators internationally. The need is first of all to provide clear information to regulators and the public about the possible hazards and the relative effects on health status.

A global index must cover every environmental compartment and must be based on detailed scientific meaning behind each hazard calculation.

However, at present global indices are not designed to produce a single value for the potential risk of anthropogenic and naturally occurring compounds to humans and the environment for each individual ecological compartment (water, soil, sediment, air).

To face this challenge the work of my PhD, detailed in this thesis, has been to create and evaluate an index: the **Environmental Risk Index for a Chemical Assessment (ERICA)**. Each element that constitutes ERICA has a strong scientific basis derived from updated guidelines and scientific data, merged into one single value within an innovative relationship. The main idea behind ERICA is to get a comprehensible picture of the general situation of a critical area; this is useful for detailed risk analysis of potentially dangerous compounds and for comparisons in time and space.

ERICA starts from an evaluation of the single compound overall risk (SRI, Substance Risk Index) (as represented in **Fig. 1**), extends it to all compounds and then assesses the entire chemical load for a specific territory.

In the context of risk assessment, ERICA can be considered as a diagnostic and prognostic tool for environmental contaminants in critical and potentially dangerous sites, for instance locations in the vicinity of incinerators, landfills or industries.

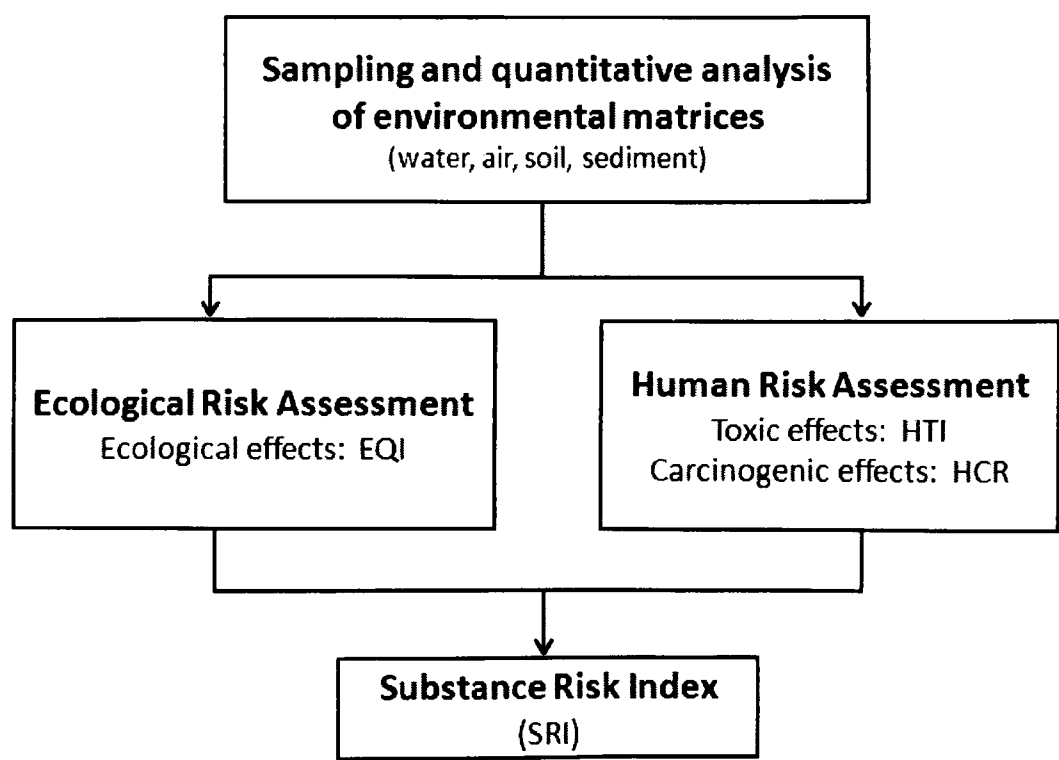


Fig.1. Information from environmental sampling and analyses are used to evaluate the impact on human (HTI and HCR) and ecological (EQI) targets. Then, the integrated results provide an overall evaluation of the risk due to the exposure to a single pollutant.

In **CHAPTER 1** an overview of the state of the art of the main international risk indices is presented, in particular a schematic system to describe diverse methods to rank and screen chemicals. The approach is first a descriptive list and summary of some of the most used systems for ranking, scoring and calculating the risk followed by a more detailed description of four systems

(SCRAM, RSEI, EURAM, USETOX), taken as examples for comparison with the ERICA index.

Then, in **CHAPTER 2** the ERICA risk index is presented, evaluating pros and cons and specific features of each equation, together with the idea and the philosophy behind the index and a description of its applicability.

The database based on the results of real analytical and quantitative data for toxic compounds on the basis of international lists and commonly found in the potentially polluted areas studied by our Environmental Health Department is presented, with a clear definition and identification of the structures of chemicals under study (name, CAS number, EINECS, SMILES, InChI, 2D structure) of each compound. Many indicators are added to describe the toxicological and ecotoxicological properties of each substance. Indicators, also called descriptors, are inherent properties of each substance (i.e. ecotoxicological, physico-chemical and fate and transport properties). They are found in the literature or calculated with dedicated reliable and validated models and software.

For example, a compound can be characterised by its molecular formula, its bidimensional parameters, partition coefficient among different environmental compartments, bioconcentration factor, ecotoxicity versus fish and soil invertebrates, toxicity versus human endpoints (carcinogenicity, mutagenicity, skin sensitivity) and so on. All these characteristics describe the compound not only for its inherent structure but intrinsically for its effect, its mode of action in the environment and possible hazard related to its concentration and speciation in the environment under study.

The index presented was created mainly to deal with effects of chemical substances in the environment. In future the exposure studies will also be part of the index.

A small number of priority compounds (19) define the minimum scenario to build ERICA. These compounds are chosen on the basis of their relevance and the relative knowledge of their toxicological profiling, their environmental distribution and anthropogenic emissions.

Therefore, the index is a simple representation of the total amount of substance and their properties in a single value. It will lead to an environmental and health risk assessment evaluation of the state of a site knowing the main substances present and their concentrations.

ERICA merges into a single number the environmental assessment, the human health risk assessment and the uncertainty due to missing and unreliable data. For this purpose, ERICA has adopted a dedicated scoring system, using parameters for relevant characteristics.

The adopted scoring system is well explained in **Appendix 3**; it is a way to weight the diverse parts and indicators that create the index in order to normalise each value and enter it into the final ERICA equation. This scoring system is an adjustment that is at the moment a categorical value but in the future could become a continuous value once the index is refined (e.g. a modern digital scale with two digits); this will lead to a system totally transparent and independent from any “a priori” decision.

Methodologies developed for Risk Assessment can be used in LCA if coherently harmonized. The ERICA index could easily be part of a more elaborate overview of risk assessment analysis because of its nature.

LCA evaluates the environmental aspect of a product system through all stages of its life cycle. As a product the energy produced by a waste incinerator plant is also considered. This Life Cycle approach or ecobalance is an emerging family of tools that is already subdivided in different degrees of complexity, depending on the type of variables and indicators considered.

In ERICA, the indicator EFI (Environmental Fate and transport Index) is also considered. It is specific for an evaluation of hazard and exposure along a timescale and it is empathised in a multiplicative factor.

A large part of the index describes the human receptors for a complete definition of risk; the human risk assessment in ERICA is divided in two main parts: toxic effects and carcinogenic effects.

Finally the equation of the index is presented associating each different element mentioned above in a overall value. The equation can be simplified with few elements or enlarged in case a large number of data are available.

CHAPTER 3 describes how the local risk for each compartment (aquatic organisms, soil organisms, birds and mammals, humans) are part of the index, considering the various organisms, endpoints, receptors specified by ecotoxicological, toxicological and fate and transport indicators. Furthermore, also the physico-chemical properties (Log Kow, etc.) are defined and exemplified for their main functions.

A large part of the work reported in Chapter 3 is related to the availability and reliability of the above-mentioned indicators. To build the index, much time was dedicated to the creation of a database, that includes all the useful indicators for each compound. The indicators derive from both literature sources (experimental model mainly) and predictive models. Due to the fact that often only a small number of consistent data are available, the main predictive models are described.

Chapter 3 presents some evaluations among experimental and predictive data that were run to compare their reliability.

Briefly the uncertainty is a typical characteristic of all data, of any nature. This refers to all values for the exposure and effect related risk assessment, plus there is the uncertainty relating to the missing data, that should be taken into account dealing with a complex scenario. There are performance values for each analysis

(*in vitro* and *in vivo*) and for each reliable predictive model. Attention should be paid in the evaluation to the total amount of uncertainty when deriving the overall definitive ERICA score.

The index is then applied to two case studies of an area surrounding a landfill and industry and Italian legislation; these cases studies are described in detail in **CHAPTER 4** within the calculation to obtain the ERICA index and the information provided.

At the end of the thesis some pages are dedicated to the **conclusions**, mainly regarding the use of the ERICA index: its strengths, its applications, and its further development.

CHAPTER 1: State of the art of freely available ranking methods for environmental and health risk assessment

1.1 Introduction

An evaluation of the important issues inherent in the development of consensus between ranking and scoring systems for a global risk assessment is presented in this chapter. Furthermore, important similarities and differences between different global systems and the index ERICA created in this thesis are evaluated.

Risk Assessment is often defined as a management tool but there are still many definitions of this term. In this thesis Risk assessment is characterized as a process of several elements including the description of potential adverse health and environmental effects based on an evaluation of results of epidemiologic, clinical, toxicological, and environmental research. Extrapolation from these descriptions is used to predict a probability that the risk will occur. Risk assessment also includes characterization of the uncertainties inherent in the process of inferring risk.

For some observers, the term is synonymous with quantitative risk assessment and emphasizes reliance on numerical results. The broader definition includes quantification, but also includes qualitative expressions of risk. Quantitative estimates of risk are not always feasible, and they may be avoided by agencies for policy reasons. Broader uses of the term also embrace analysis of perceived risks, comparisons of risks associated with different regulatory strategies, and occasionally analysis of the economic and social implications of regulatory decisions—functions that in this thesis will be defined as *risk management*.

The term *risk management* is used to describe the process of evaluating alternative regulatory actions and selecting among them. Risk management,

which is carried out by regulatory agencies under various legislative mandates, is an agency decision-making process that entails consideration of political, social, economic, and engineering information with risk-related information to develop, analyze, and compare regulatory options and to select the appropriate regulatory response to a potential health or environmental risk. The selection process necessarily requires the use of value judgments on such issues as the acceptability of risk and the rationality of the costs of control. (Van Leeuwen and Vermiere, 2007; NAS, 1994; RAGS- Risk Assessment Guidance for Superfund, 2001),

At the present level of understanding it is still difficult exactly to predict adverse effects on ecosystems, or what section of human population will be affected. As reported in many tests related to risk assessment (e.g. Van Leeuwen et al., 2007) it is possible to assess risk only in a very general and simplified manner. The preferred approach is to provide a relative risk ranking. Relative risk ranking allows for understanding which process, technique, chemicals has to be replaced or monitored in the risk management phase without knowing the precise risk.

1.2 Risk Assessment, ranking and scoring systems – a brief overview

-

Risk can be defined as the probability of an adverse effect in an organism, system or (sub) population caused under specified circumstances by exposure to an agent (IPCS and OECD, 2003).

Risk management is the process of taking decisions to reduce risks according to risk assessment information but also to additional factors such as economics, technical feasibility, public policy, and/or stakeholders' considerations. The risk management process is based on the 8 main steps reported in Table 1.1 plus further possible development as reported by Van Leeuwen et al. (2007).

Table 1.1: Main points in risk managements process, including the further possible development according to Van Leeuwen et al. (2007).

Hazard identification (step 1)
Exposure assessment (step 2)
Effects assessment (step 3)
Risk characterization (step 4)
Risk classification (step 5)
Identification and risk-benefit analysis of risk reduction options (step 6)
Risk reduction (step 7)
Monitoring and review (step 8)
Focus on risk reduction and responsible care (step 9)
Risk communication and stakeholder participation (step 10)
Risk assessment policy and the role of science (step 11)
Integration in risk assessment (step 12)

In this context risk assessment, ranking and scoring systems, and evaluation of the scientific data, are all factors that may substantially vary the outcome of the decision making process in the risk management process.

Chemical screening in the United States is often conducted using scoring and ranking methodologies. Linked models accounting for chemical fate, exposure,

and toxicological effects are generally preferred in Europe and in product Life Cycle Assessment.

An LCA process is sometimes defined in such a general way that is difficult to “translated” to a simple ranking for the risk assessment process because the toxicity and effects of the chemicals (as for example CO₂) is measured in toxicity equivalent, and also between LCA equivalent data there is controversy depending on the target of the analysis (environment, human, water surface, industrial product).

But at the same time all LCA methods have the ability to merge within a large scheme several layers, in which the ranking of chemicals of interest and their impact in the environment are considered two of the most important steps.

In the work presented in this chapter a models built for LCA (Life Cycle Assessment) is also described because often LCA methodology is very good to propose a first ranking of hazardous chemicals in the environment even if often it is aimed to be applied at larger scales, mainly to assess industrial systems, the so called “cradle to the grave” process.

“Cradle-to-grave” begins with the gathering of raw materials from the earth to create the product, and ends at the point when all materials are returned to the earth. LCA evaluates all stages of a product's life from the perspective that they are interdependent, meaning that one operation leads to the next. So, in a few words LCA enables the estimation of the cumulative environmental impact resulting from all stages in the product life cycle, often including stages not considered in more traditional analyses (e.g., raw material extraction, material transportation, ultimate product disposal, etc.). By its inclusion throughout the product life cycle, LCA provides a comprehensive view of the environmental aspects of the product or process and a more accurate picture of the true environmental trade-offs in product and process selection.

A very good review about the various LCA models available is presented in the website: <http://www.epa.gov/nrmrl/lcaccess/>.

Cumulative Risk Assessment indicates a risk assessment where multiple stressors and exposure ways are considered. In other words it is an analysis, characterization, and possible quantification of the combined risks to health or the environment from multiple agents or stressors. Stressor is defined as any physical, chemical, or biological entity that can induce an adverse response. A stressor may also be the lack of an essential entity, such as a habitat.

Health risk assessment and ecological risk assessment have been developed largely independently. Current practices in health risk assessment have derived largely from the framework developed by the U.S. National Research Council (1983). Current practices in ecological risk assessment have largely derived from the framework developed by the U.S. Environmental Protection Agency (1992).

These separate frameworks have been associated with separate risk assessments, performed by separate assessment teams, using largely separate data, models, and assumptions.

The World Health Organization's International Program on Chemical Safety (WHO/IPCS), in collaboration with the U.S. Environmental Protection Agency (EPA) and the Organization for Economic Cooperation and Development (OECD), has developed a framework for integrated human health and ecological risk assessment (WHO, 2001; Suter et al., 2003).

The integrated risk framework was developed for two fundamental reasons (1) to improve the quality and efficiency of assessments through the exchange of information between human health and ecological risk assessors, and (2) to provide more complete and coherent inputs to the decision making processes (Suter, 2005).

Global Risk Assessment, as it is intended in the present work, is an integrated risk assessment strategy able to include also the fate and transport parameters of compounds.

Human health risk assessment involves the evaluation and quantification of potential health hazards to humans from exposure to substances and agents in their environment.

Since the end of the 1990s, it has been common for health risk assessment to adopt a paradigm, first developed by the National Academy of Science (NAS, 1994) for human health risk assessment and later also adopted for environmental risk assessment (USEPA, 1992). NAS identified four steps for risk assessment: Hazard Identification/ Dose-Response Assessment -Effect Assessment/ Exposure Assessment/ Risk Characterisation.

In the Hazard Identification step, one identifies the contaminants that are suspected to pose health hazards, quantifies the concentrations at which they are present in the environment, and describes the specific form of toxicity. Then, in the Effect Assessment Step, there is an evaluation of the condition under which these forms of toxicity might be expressed in exposed humans. The data required in this step come primarily from scientific literature from the field of toxicology and epidemiology. The third step (Exposure Assessment) is focused on the determination of emissions, pathways and rates of movement of a chemical with the aim of estimating the concentrations or doses to which human or ecological targets are exposed, through the application of fate and transport or other mathematical model or through direct environmental measurements.

Finally, in the Risk Characterisation step, the information about exposure and dose-response relationships are integrated in order to achieve an estimation of the incidence and severity of the adverse effects likely to occur in the considered targets. There are many variables involved at this step to describe and to interpret the available data and estimate the risks. Until a few years ago expert judgment in this step was essential, but now an objective, transparent and

common protocol to deal with all risk assessment steps is being developed. Furthermore, working groups like the Numerical Unit Spread Assessment Pedigree (NUSAP) (van der Sluijs et al., 2005) are dealing with the matter of including expert judgment in risk assessment and a protocol (reference to "Pedigree Systems") has already received peer review to be part of future risk assessment processes.

Uncertainty factors are numerical factors applied to a toxicological reference point to allow for uncertainties in risk assessment. These factors may be default values used in the absence of specific information on a chemical and may be modified in the light of specific information.

While for human health risk assessment a standardized, scientifically based operational procedure has been developed and is quite widely accepted internationally (USEPA, 1989), no operative standard procedure yet exists for environmental risk assessment, but there are various different methodological proposals reflecting the great variability of the different sensitive targets (Ruden et al. 2010) .

Often in the characterisation of risk so-called indicators and indices are used; in general the development of indices and indicators built for a specific contest has given more reliable answer to assess risks. Several risk indices are based on reliable indicators, for instance of toxicity or physico-chemical properties.

Several indicators for reporting environmental and human health conditions have been published (UNCDS, 2006) and indicator frameworks have also been published for chemicals (Bunke and Oldenburg, 2005), hazardous wastes (Peterson et al., 2001; Peterson and Granados, 2002) and hazardous materials at landfill sites (Peterson, 2002). Some are indicators for chemical management using an analytical framework based on a life-cycle approach (DEFRA, 2010), while others are concerned with pollutant chemical releases (DETR, 1999; Eurostat, 2008), risk screening for chemical releases (USEPA, 2004), impact of chemical emissions (EEA, 2007) and chemical sources.

At present, new methods for risk assessment of chemicals for both human and ecological targets are being developed, in particular in Europe after the implementation of REACH legislation. For example the EUSES method is a decision-support instrument, which enables government authorities, research institutes and chemical companies to carry out rapid and efficient assessments of the general risks posed by substances to man and the environment. EUSES is intended mainly for initial and refined risk assessments rather than global assessments. Programs like EUSES generate frameworks with equations useful to simplify the calculation of the risks but the decision to be taken to conduct the analysis requires the expert knowledge, and it may lead to different conclusions dependent on risk management choice (EUSES, 2008).

In parallel, a number of research organizations have started projects to analyze the “state of the art” of pesticide risk indicators, to examine the outcome and limitations of different approaches and to harmonize the use of these indicators internationally. For instance, the EU CAPER (Concerted Action on Pesticide Environmental Risk indicator, 1999) project compared eight indicators developed for various purposes and built using different approaches for risk evaluation. The Organization for Economic Cooperation and Development (OECD) also carried out projects on pesticides focusing mainly on the analysis and development of indicators for governments. The indicators should track temporal risk trends in agricultural pesticide usage on different geographical scales (field scale, regional scale, national scale) and should follow up the progress in meeting pesticide reduction goals. HAPERITIF (Calliera et al., 2006) is one of these indicators for monitoring pesticide risk trends attributable to dietary pesticide exposure on various geographic and temporal scales, while ERIP (Finizio et al., 2001) and EPRIP (Trevisan et al., 2002) are related to the ecotoxicological effects in soil. For pesticides the assessment is improved by a greater number of data for both the effects and the exposure scenarios. Pesticides offer useful examples since in the last few years several indicators have been developed and applied in different EU countries, aiming at different goals. Some indicators have been

developed as decision support system tools, to assess the potential environmental or economic consequences of pesticide management systems. Others are intended to encourage more sustainable crop production or are applied in assessments for granting ecolabels. Finally, other indicators monitor temporal risk trends on different scales.

Evaluating ecological risk is complex, since it requires detailed knowledge of the biotic and abiotic components of the considered ecosystem, in order to obtain a realistic estimate of all the exposure pathways of the contaminants. Such an approach is not only very expensive in terms of the use of human, economic and time resources, but it also needs to be supported by the integration of different scientific areas. An important step in this direction should be made concerning the prediction or the calculation of the fate and transport (FT) data of some chemical classes, since FT varies widely depending on the site-specific pedologic, hydrogeologic and meteoroclimatic conditions.

1.3 Comparative tools

There are at present few exhaustive and recent reviews and comparative works in the literature on methods for global risk assessment and screening procedures. The main reason for the limited number of comparisons is that every method is built for a specific purpose and in this context it is not possible to analyse the possible use and applicability of each method.

This introduction aims to give an overview of available tools/models comparable to ERICA, and then to underline the characteristics of each system in a simple and schematic way.

First of all it is good to remember that although numerous ranking and scoring systems have been or are being developed, there is currently no scientific consensus on risk ranking methods. Chemical risk ranking has received the most attention, and several systems have been used, for example, to determine which

chemicals should be included in various regulatory pollutant lists. To facilitate development of a framework for overall human health and environmental risk ranking, some reports presents criteria to judge the reliability of the methods.

Among these reports two of them present important methodologies: the first is a comparison done between The Waste Minimization Prioritization Tool versus Toxic Equivalency Potentials (Pennington, 2001) and the other is the EPA Guidance "Comparative evaluation of chemical ranking and scoring methodologies" (Swanson et al., 1994).

The first is focused mainly on comparing two of the prominent but structurally different methodologies adopted to help screen and rank chemicals and chemical emissions data.

The scope of the comparison was restricted to human health, although the insights would be equally useful in the context of the health of ecosystems.

Within this comparison, current types of chemical screening and emissions comparison approaches are illustrated and the relative significance of the scenario and structural differences of the Waste Minimization Prioritization Tool (WMPT) and the Toxic Equivalency Potential (TEP) methodologies are analyzed. The WMPT facilitates comparisons in terms of key physico-chemical properties, Persistence, Bioaccumulation and Toxicity (PBT). Each PBT measure is scored and then these scores are added to provide a single measure of relative concern. TEPs account for chemical fate, multipathway exposure, and toxicity using a model-based approach. This model structure is sometimes considered to provide a less subjective representation of environmental mechanisms, and hence an improved basis for screening. Nevertheless, it is claimed that a strong relationship exists between the two approaches, and both have their limitations.

In the EPA Guidance a possible way to evaluate the different methods taking into account some specific elements is presented:

- the purpose and application of the ranking and scoring system;

- the human health criteria and endpoints included in the assessment;
- the criteria and endpoints included for environmental effects;
- whether measures of exposure are included;
- the approach used for data selection and for handling missing data.

1.4 Aim of the analysis done on ranking and scoring systems for a global risk assessment

This chapter lists some of the most used ranking and scoring systems followed by a more detailed analysis of four systems chosen for their flexibility and availability, i.e. SCRAM (Snyder et al. 2000a,b,c,d), RSEI (USEPA, 2009), USEtox (Rosenbaum et al., 2008), ERICA (Boriani et al., 2010).

The analysis is structured as a comparison of the selected system according to several criteria/elements which allows one to consider in a single picture (Table 1.2) similarities and differences among the systems and to facilitate the comparison and evaluation of strengths and weaknesses of the evaluated systems.

The strategy adopted to develop the evaluation method includes the analysis of the following elements:

- a) the criteria to include classes of chemical in ranking and scoring system;
- b) the endpoint(s) used to measure or score those criteria;
- c) data selection approaches to be used to select or estimate data for the criteria;
- d) how should criteria scores be weighted and combined to reach an overall score or rank for each specific chemical (if an overall score per chemical is the goal);

- e) what level of sophistication in effect and exposure estimation is appropriate - from total amounts produced, used or released, to multimedia environmental fate models, to site-specific models including estimates of dose over time;

Reassembling these elements into one framework, providing flexibility to use some or all of the components for specific purposes goes beyond the aim of this chapter. Here I have built up a simplified scheme aimed at highlighting the differences among the methods.

Of course on a larger scale, as claimed in the two above mentioned papers (Pennington, 2001; Swanson, et al., 1994), the process of developing the consensus framework should involve all of the significant stakeholders - government agencies, chemical manufacturers and users, environmental and consumer groups, and academic researchers. This will create greater acceptance of the results and a good base for risk management.

Table 1.2 Summary of model comparison. Legend: green= strength, yellow= limitations,

(-)= missing information, (+)= minimum information, (++)= acceptable information, (+++)=

high quality information

	SCRAM	RSB	USBox	ERCA
PURPOSE AND APPLICATION	Water compartment – Evaluation of PBT- ranking for risk assessment	Screen Level – GRD based – Human toxicity	Global LCA – World scale model- ecotox/human/physico-chemical – characterization factors	Single value determined by detailed risk assessment analysis ecotox/human/fate and transport
CHEMICAL SELECTION APPROACH	The list of chemicals (40) then scored by SCRAM comes from International Joint Commission Great Lakes Water Quality Agreement (GLWQA)	TRI (Toxicity Release Inventory), data on the quantity of chemicals released to AIR, WATER, on site disposal facilities	Industrial compounds released in the environment, characterization factors has to be computed using various parameters composed in matrices	available database of 200 compounds, (toxicity- ecotoxicity and fate and transport properties), ERCA is built on a scenario of 19 compounds that can be varied in dependence of the task of the analysis
SCORING FORMULA	geometric mean (sum of final chemical score and the final uncertainty score – missing or availability of information)	grid related to population living in the site under study, additional statistical information	set of scale-specific characterization factors (cases/kg emitted) built for exposure, effects for human and ecotoxicity	System of peer reviewed scoring systems for each endpoint used in the equations
MEDIA CONSIDERED	Compounds released in water and distributing in biota, air, water, soil, sediment	air, surface water	Continental scale, freshwater, coastal marine, industrial soil, natural terrestrial, agricultural soil, rural air, urban air	water, soil, sediment, air
HUMAN HEALTH CRITERIA AND ENDPOINT INCLUDED	(-)	non cancer, cancer	non cancer, cancer	non cancer, cancer
ENVIRONMENTAL EFFECTS CRITERIA AND ENDPOINT INCLUDED	Persistence Bioaccumulation Ecotoxicity	(-)	ecotox impact (geometric mean of acute aquatic EC50)	most sensitive species, water-soil-sediment-air, as Technical Guidelines for Ecotoxicological Risk Assessment
FATE AND TRANSPORT EFFECTS CRITERIA AND ENDPOINT INCLUDED	few use of Mac Key Level and III, persistence data calculated for the compartments where major portion of compounds were	(+)	fate and transport in world scale	Mac Key Level I and II, to calculate % of compounds in each media, physico chemical properties considered
LEVEL OF SOFISTICATION ON EFFECT	(+++)	(+)	(+)	(+++)
LEVEL OF SOFISTICATION ON EXPOSURE	(+)	(+++)	(++)	(+)
USE OF TOTAL AMOUNT OF PRODUCED-USED-RELEASED COMPOUNDS	(++)	(+++)	(+++)	(+)
USE OF ENVIRONMENTAL CONCENTRATION	(+)	(+)	(+)	(+++)
AVAILABILITY OF USED DATABASE	(++)	(++)	(++)	(+++)
POSSIBILITY OF ADDITION OF NEW CHEMICALS	(++)	(++)	(+)	(++)
INCLUSION OF MULTIMEDIA ENVIRONMENTAL FATE MODELS	(+++)	(+)	(+++)	(++)
INCLUSION OF SITE SPECIFIC MODELS	(+)	(+++)	(+)	(+)
INCLUSION OF PREDICTIVE MODELS	(+)	(+)	(++)	(+++)
QUANTITATIVE HEALTH RISK ASSESSMENT	(+)	(+)	(+)	(++)
QUANTITATIVE ENVIRONMENTAL RISK ASSESSMENT	(+++)	(+)	(+)	(++)
QUANTITATIVE FATE AND TRANSPORT	(++)	(+)	(+)	(++)
INDEPENDENCY FROM EXPERT JUDGEMENT	(+)	(++)	(+)	(++)
UNCERTAINTY PARAMETERS	(++)	(+)	(+)	(++)
COMMUNICATION OF OUTPUTS	(++)	(+++)	(+)	(+++)
strength	(-) missing information	(++) acceptable information		
limitations	(+) minimum information	(++) high quality information		

Some scoring and ranking systems have been adopted by authorities and regulatory centres mainly as first screening tools to identify the chemicals with greatest potential for adverse effects. For instance, the **SCRAM scoring and ranking assessment model** (Snyder et al. 2000 a,b,c,d) is one of these and one of the few systems that also takes the uncertainty into account when data are missing at some steps.

The SCRAM system was created after the International Joint Commission Great Lakes Water Quality Agreement (GLWQA) recommended that a selected group of chemicals (mainly pesticides and industrial compounds) should be virtually eliminated or have their existing levels in the Great Lakes basin measurably reduced. However, it was unclear which chemicals were of most concern for the Great Lakes. SCRAM was then developed, along with other methods, to assist in the review of a large lists of chemicals for the purpose of defining and ranking their relative risk. SCRAM provides a prioritization tool for risk assessors and managers to determine the concern posed by substances that are likely to be found in the Great Lakes.

North American agencies have agreed that approximately 40 chemicals can cause deleterious effects if released into the environment (Snyder et al., 2000a). Therefore SCRAM is limited to chemicals found in the environment, because its aim is mainly to screen and order chemicals based on their profile of persistence, bioaccumulation and toxicity. SCRAM provides a method to evaluate and score the persistence, bioaccumulation, and toxicity of chemicals, resulting in a 'chemical score' that integrates these three important chemical characteristics. SCRAM scores the uncertainty of the information available for each category (persistence, bioaccumulation, toxicity), thereby allowing also the assessment of those chemicals for which there are limited data. This important feature has a key role in the interpretation of SCRAM final scores (Snyder et al., 2000b,c) . The numerical ranking does not provide a measure of hazard or risk.

Before REACH legislation, in order to provide a legal framework within the European Union (EU) for the evaluation of existing chemicals, a Council Regulation (EEC) 793/93 was adopted, in which the evaluation of the existing chemicals was carried out by four steps, namely data collection, priority setting, risk assessment, and, if necessary, risk reduction.

At the time, to fulfil the priority-setting step, the EU Risk Ranking Method (EURAM) was developed to produce rankings at the basis for drawing up lists of substances, used for priority setting, among the so-called high production volume chemicals appearing in the International Uniform Chemical Information database (IUCLID). The EURAM uses a simple exposure–effect model, containing human health and environmental effect endpoints as well as exposure parameters. The EURAM has been applied and used as a basis for selecting substances for the second and the third list of priority substances as foreseen under Council Regulation (EEC) 793/93.

Among the recent screening systems, the **EPA's Risk-Screening Environmental Indicators (RSEI Version 2.2.0)** (USEPA, 2004) is a screening-level tool that assesses the potential impact of industrial releases from pounds-based, hazard-based, and risk-related perspectives. RSEI uses risk concepts to quickly and easily screen large amounts of Toxics Release Inventory (TRI) data, saving time and resources. RSEI is particularly useful to examine changes in chemical production and releases, to rank qualitatively and to prioritize chemicals and industry sectors for strategic planning, to conduct risk-related targeting, and supporting community-based projects.

The **USEtox** (Rosenbaum et al., 2008) model is included in the analysis presented in this chapter as an example of a LCA model. It is an environmental model for characterization of human and ecotoxic impacts in Life Cycle Impact Assessment and for comparative assessment and ranking of chemicals according to their inherent hazard characteristics. The USEtox™ model has

been developed by a team of researchers from the so called “Task Force on Toxic Impacts” under the UNEP-SETAC Life Cycle Initiative.

1.5 Results

For some of the mentioned models, i.e. SCRAM, RSEI, USEtox, ERICA, a detailed comparison has been performed, and results are reported in this paragraph. Table 1.3 describes some of the main features of the models considered in the analysis. The purpose of the table is to summarise the differences and diverse abilities of each model. In particular the following elements were taken into consideration while describing the diverse models.

PURPOSE AND APPLICATION: Each model was built for a specific task and, accordingly, it can be applied on a particular scale (local scale, regional scale, global scale).

CHEMICAL SELECTION APPROACH: Some models were built appropriately to rank chemicals of concern already listed by authorities, (e.g. SCRAM) while other have their own list of chemicals of concern (e.g. ERICA) defined on the basis of expert judgment as the most commonly found in the environment others put together diverse databases to assess a global ensemble of industrial releases (e.g. RSEI and USEtox).

SCORING METHOD/SCORING SYSTEM: The scoring formula is a way to transform the data part of the models in more easily managed values. It is the method used to normalise the diverse indicators to further their being aggregated. For some models with few parameters (e.g. SCRAM that deals only with PBT) the geometric mean is used and another scoring value is used for an uncertainty parameter in case of missing or unreliable values. Other models (e.g. ERICA) have their own scoring system using information from peer-reviewed sources of information.

MEDIA CONSIDERED: The media considered are in some models the environmental compartments where the contaminants have been found, and in others the environmental compartments also, where contaminants diffuse and migrate; in other words some models consider only the compartments where the compounds are found while others considers also the percentage of compounds that will migrate and diffuse in to another medium (e.g. SCRAM considers water compartment and diffusion from water to other compartments, while ERICA considers the concentration of contaminants in sediment, air, soil and water compartments but also the diffusion of each chemicals in the various compartments).

The following three points:

EFFECTS ON HUMAN HEALTH CRITERIA AND ENDPOINT INCLUDED

EFFECTS ON ENVIRONMENTAL CRITERIA AND ENDPOINT INCLUDED

EFFECTS ON FATE AND TRANSPORT CRITERIA AND ENDPOINT INCLUDED

simply list and describe synthetically the criteria used in the model to judge the effects on the various endpoints taken into consideration.

The bottom part (last 14 rows) of Table 1.3 refer to other features of the evaluated model which have been considered important in the comparison. In the table these features are judged for each model using a code (Table 1.4) to report how much information is provided for each aspect/element. The sources of information are the manuals provided within the models, the model itself and the papers or reports describing or concerning the models.

This simple description aims to give an easy overview of each model features.

Table 1.3 Main features of the models considered in the analysis

LEVEL OF SOPHISTICATION ON EFFECT

LEVEL OF SOPHISTICATION ON EXPOSURE

USE OF TOTAL AMOUNT OF PRODUCED-USED-RELEASED COMPOUNDS

USE OF ENVIRONMENTAL CONCENTRATION

AVAILABILITY OF USED DATABASE

POSSIBILITY OF ADDICTION OF NEW CHEMICALS

INCLUSION OF MULTIMEDIA ENVIRONMENTAL FATE MODELS

INCLUSION OF SITE SPECIFIC MODELS

INCLUSION OF PREDICTIVE MODELS

QUANTITATIVE HEALTH RISK ASSESSMENT

QUANTITATIVE ENVIRONMENTAL RISK ASSESSMENT

QUANTITATIVE FATE AND TRANSPORT

INDEPENDENCE FROM EXPERT JUDGMENT

UNCERTAINTY PARAMETERS

COMMUNICATION OF OUTPUTS

Table 1.4 Code used to report how much information is provided for each aspect/element

(-)	absence of information
(+)	minimum information
(++)	acceptable information
(+++)	high quality information

LEVEL OF SOPHISTICATION: This parameter indicates the number of processed information to determine effect and exposure models. In some cases simple/ immediate quantities are used (e.g. in SCRAM in case of missing data on BCF only LogKow is used instead of the use of a more complex predictive model or in other case is missing an in depth evaluation of the variability of test data). In other cases an in depth study is conducted before calculating the effect or exposure. For example, the epidemiological study, conducted in situ, is robust in RSEI and this leads to good parameters for exposure. In ERICA the level of sophistication on effect is high because it covers several steps (variability of experimental data, comparison of predictive methods, use of the most reliable assessment factors, etc)

EXPERT JUDGMENT: This parameter highlights whatever the opinion of an expert has a important role in the model or not, in other words if the model requires some specific information at some stages, which could be provided only by an expert, or if the model is structured to be usable by everyone.

UNCERTAINTY PARAMETERS: in the risk assessment process, the major sources of uncertainties in brief are: lack of information regarding toxicity data,

imprecise data on emission and exposure, fate and transport behaviour of chemicals, uncertainty factors used to adapt models (interspecies, intraspecies, experimental models, predictive models, in vitro models), models and simplified equations to represent the reality using hypothesis and simplifications. In the considered ranking/scoring models, sometimes the uncertainty is defined in the various steps of the models through a score (e.g. in the SCRAM model). In other cases, uncertainty is an unknown parameter for some endpoints, in which case it is not considered in the final result for its potential complexity (e.g. USEtox). In other models (e.g. ERICA) it is considered as a parameter to be minimised in each step of the risk assessment process, mainly in the choice of the data (toxicological, ecotoxicological, fate and transport information). Uncertainty is a complex parameter having many diverse aspects, and in the reported classification all the type of uncertainties are taken into consideration.

COMMUNICATION OF OUTPUTS: Some software results are very easy to understand since they give simple outputs that easily explain the risk to the population and risk assessors (e.g. ERICA model final output is a single number easy to interpret also if it is the final result of a series of interdependent equations). Other models' results may be more difficult to interpret because there are many parameters to be read to provide an overall interpretation of the output (e.g. USETox output has to be interpreted by experienced people who gained insight on LCA data in order to understand the dimensions of parameters and their importance in the context of the industrial process considered).

1.6 Discussion and conclusions

As a result of the analysis performed on the selected systems, it is possible to summarize some strengths and some limitations for each model, represented by the code colour reported in Table 1.2. The limitations are coloured in yellow (code – and code +) and the strengths of each model (+++) are coloured in green. The code (++) symbolizes that, according to the manual and the results,

the model processes that parameter but does not give the best performance using it. The applicability domain of the models is easily perceived checking the results table and the description of the abilities of each model. This easy comparative method could support practically the choice of one model against another one, depending on the desired output and taking into account the context of the analysis.

Some models can be viewed as complementary, and joint application could be considered, as the overall results may lead to better performances.

CHAPTER 2

ERICA: a multiparametric toxicological risk index for the assessment of environmental healthiness.

In this chapter the ERICA index is presented. Before the theory a diagram (Fig 2.1) is shown to help the reader follow the process of building up the index.

Further references regarding this work are in Appendix 3, in the paper “ERICA: a multiparametric toxicological risk index for the assessment of environmental healthiness.” (Boriani et al. 2010).

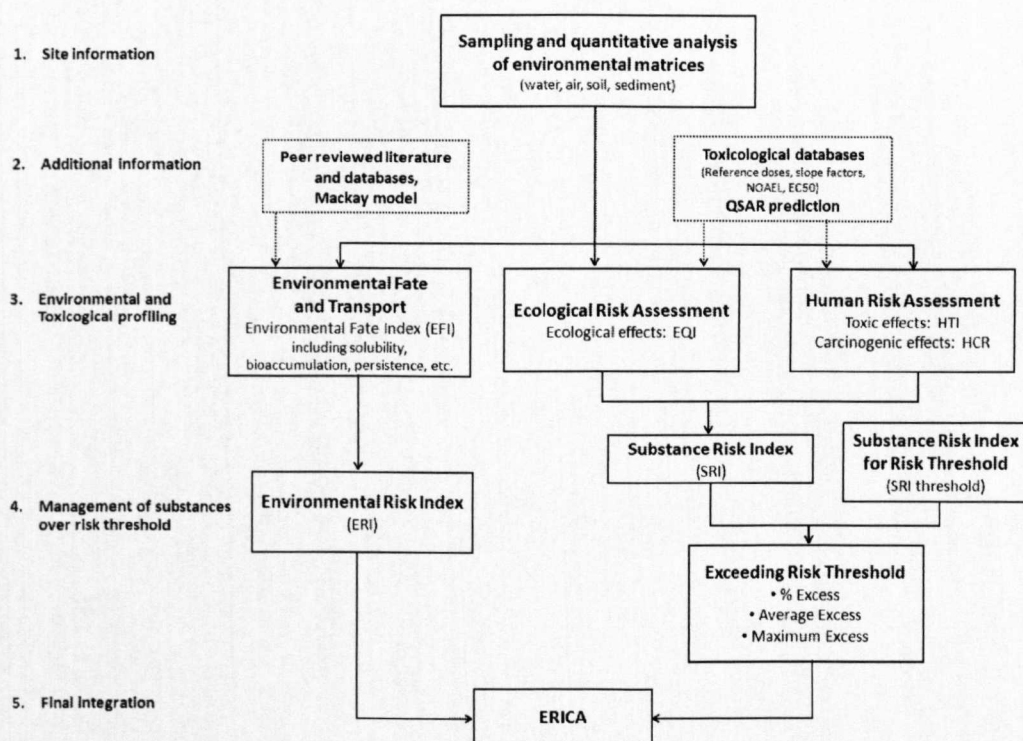
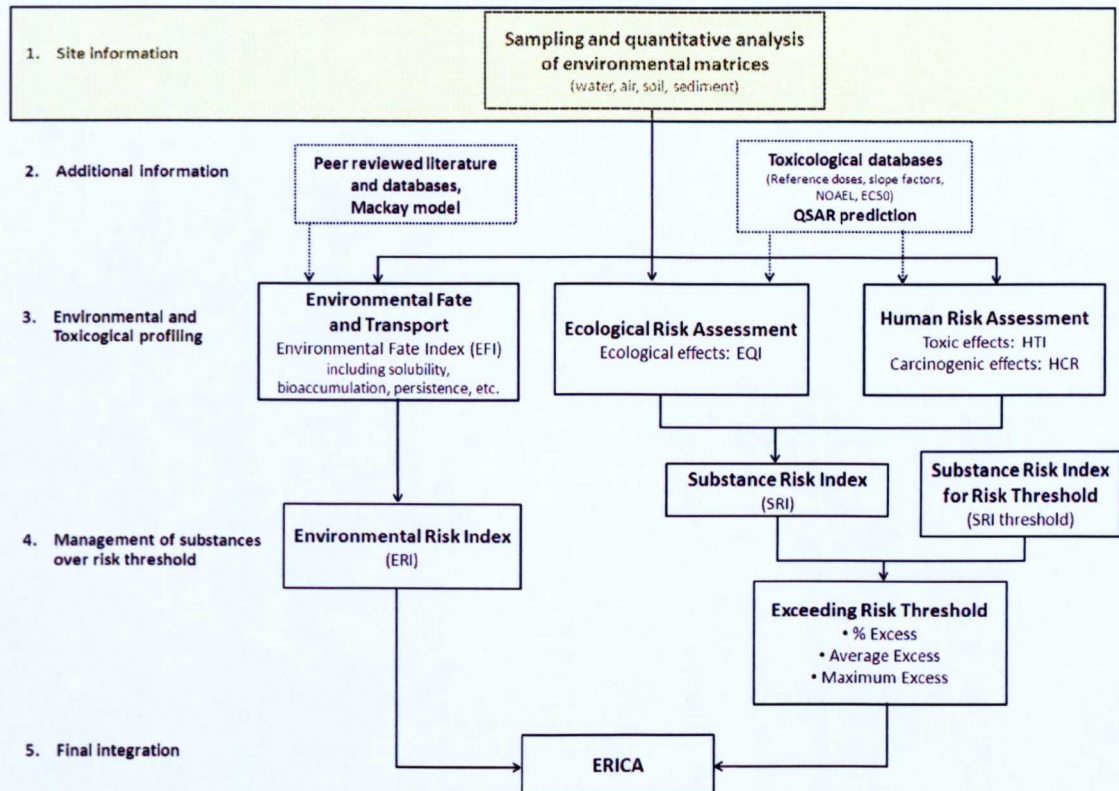


Fig. 2.1 ERICA integrated flowchart: ERICA has three main parts: A Human Risk Index (consisting of HTI: Human Toxicological Index and HCR: Human Carcinogenic Index), an Environmental Toxicological Index (EQI) and the Environmental Fate and transport Index (EFI). The environmental risk index (ERI) is weighted from the EFI for each compound. The overall Substance Risk Index (SRI) is a balanced addition of these indices. Risk limit is used for the compounds without data on environmental concentrations. The approach for each selected pollutant is shown in the upper part while the integration process for the overall effects is reported in the lower part.

2.1 Materials and methods

2.1.1 Site Information (Part 1 in Fig 2.1):



To apply ERICA on a real environment site it is necessary to have specific information regarding the concentration of pollutants in the main matrices: water, soil, air and if it is available also sediment. Of course this information depends on the places under study and may vary a lot in dependence on the type of the qualitative or quantitative analysis that can be or are already conducted at the site under study. Some practical evaluation of risk, done by our institute in some specific territory located nearby landfill or incinerators or industrial areas and then evaluated with ERICA index, are presented in Chapter 4.

It is not always possible to gain all the data necessary to run the ERICA index because of the cost of the relative analysis or time to conduct the analysis or the

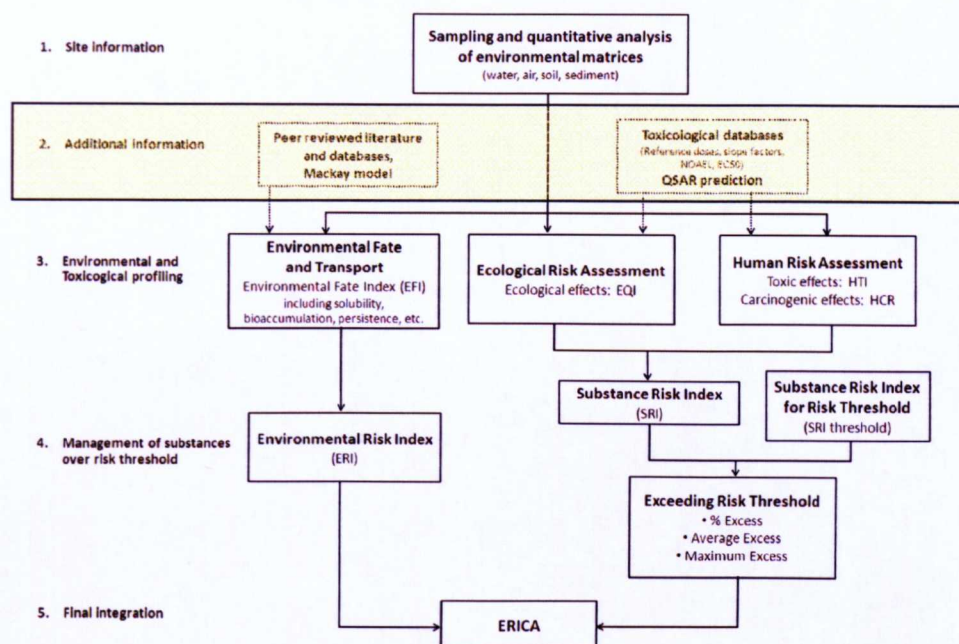
privacy of some information and in these cases the missing information is evaluated in a conservative way. A conservative way means that the maximum risk pose by the missing substances is taken. In case an entire compartment is missing it is possible to relate ERICA only to the other compartments, and often this simplified way is useful to compare site with only, for example, information on soils.

It is necessary to get information on the substances of maximum concern and it may happen that a particular site has potentially dangerous compounds not present in another site. In this case, instead of the scenario set of compounds presented here, it is possible to choose a set of compounds that better represent the area under study.

A database of 186 compounds was built to provide a broad basis to ERICA and its structure is explained in the next paragraph.

2.1.2

ERICA Database structure (Part 2 of the Fig 2.1)



The ERICA database of 186 chemicals includes their toxicity and physico-chemical properties from referenced experimental data or reliable predictive methods.

An excel file containing the database in its latest version is available with the present work (**Appendix 1**). The database is constantly updated with new experimental and predicted data available.

The list of potentially dangerous compounds in the database focuses on these main chemical classes:

polycyclic aromatic

hydrocarbons (PAHs),

chlorobenzenes,

nitrobenzenes,

phenols,

chlorophenols,

halogenated aliphatic hydrocarbons,

polychlorinated dibenzodioxins (PCDDs),

polychlorinated dibenzofurans (PCDFs),

polychlorinated biphenyls (PCBs),

pesticides,

hydrocarbons and

inorganic compounds (e.g. metals, ozone, carbon monoxide).

Other compounds will be added when data on their physico-chemical properties and toxicological profiles are available from international databases, peer-reviewed literature or from QSAR applications.

Using the molecular names and/or CAS (Chemical Abstract Service) numbers, the two dimensional (2D) chemical structures for each compound were checked in five online databases (HSDB, TOXNET, CHEM ID Plus, ChemFinder, PubChem, Safe Nite). 14 substances also required in regulatory risk assessments (i.e. fluorurate classes or particulate matter (PM₁₀, PM_{2.5})), are mixtures of chemicals. Thus, it has not been possible to define a single structure and the properties from the literature were directly reported in the database, while for the other cases the relative structure is indicated. This fact also allowed the use of predictive models where experimental data were missing or unreliable.

Much attention is nowadays focused on these methods (as reported in CHAPTER 3) to avoid experimental tests on animals because of cost, time and ethical problems. To use predictive models it is fundamental to start with a well-

defined input and structure, because descriptors related to that structure will drive the structure-activity relationship that is the basis of predictive models.

To fulfil the best description of a structure careful evaluation of many sources of data are important to compare them and to critically check if the results of the prediction make sense.

A structure-data file (.sdf) containing various types of information related to the substances (ID, SMILES, CAS number, 2D structures, main physico-chemical properties) was created for modelling purposes and is also available within the present work (Appendix 1).

In Table 2.1 the information available for each compounds present in the database is summarised.

Table 2.1 Information for each compound contained in the excel data file

Identifier	Physico-Chemical properties	Distribution parameters	Ecotoxicological data	Toxicological data
Chemical ID, Name, CAS number, SMILES	MW, Solubility, Biodegradation (Biowin model), Koa, Kow, Kaw, Koc, Vapour Pressure, BCF, BAF	Mackay Model Level I, Mackay Model Level III – Fugacity model	Acute inhalatory toxicity, acute oral toxicity, acute water toxicity	Class of carcinogenicity, slope factor for ingestion, slope factor for inhalation, reference dose for ingestion.

2.1.3 The minimum scenario

19 priority substances have been selected for their toxicological profiling, frequent environmental occurrence or common presence in anthropogenic emissions. Recent prioritization systems from authorities (e.g. Stockholm Convention on Persistent Organic Pollutants, Toxic Chemical Release list (USEPA, 2006)) were taken into account in choosing these compounds.

They reflect a minimum, heterogeneous scenario that covers all four environmental compartments (water, soil, sediment and air) considered in ERICA, and are well spread out.

The selected pollutants are the following:

As,

Cd,

Cr,

Hg,

Ni,

Pb,

benzene,

PAHs as benzo(a)pyrene equivalent,

PCDD/PCDFs as 2,3,7,8-TCDD equivalent,

PCBs as 2,3,7,8-TCDD equivalent,

NO₂,

SO₂,

CO,

O₃,

PM₁₀,

PM_{2.5},

DDT,

atrazine,

hexachlorobenzene

They represent the so called "minimum scenario" that has to be considered for each compartment (soil, water, air, sediment) and endpoint (environment, human toxicity, human carcinogenicity) while calculating the index for a case study.

For scenarios where some experimental data are missing the hazard limits are used. Hazard limits, here called risk thresholds, are the limit values at which the risk will start to occur.

The reason for the minimum scenario is that it is useful to get a general idea of the status of a considered area with a comparable representation for different plants or situations.

In different countries or for different tasks the minimum scenario can be modified. This will produce only a relative change in the final ERICA equation because the final ERICA value is related to the emerging risk posed by each single compound exceeding the hazard limit.

The hazard limit could be easily defined as the limit when an adverse effect starts to occur for any endpoint. This limit is different from the legislative limit that is a compromise related to the risk management process. In CHAPTER 4 the case

study of the Italian law limit scenario is presented and shows an adverse effect for sensitive organisms. In fact law limit values are often used in the risk assessment analysis but have the limitation of not covering all the hazard compounds. Furthermore the legislative limits have been shown to be higher than the concentration causing adverse effects on the most sensitive species.

The proposed scenario in ERICA should be extended with other compounds depending on the preliminary characterization of the investigated site: information available, industries emissions, site-specific history of previous contaminations, epidemiological evidence and ecological evidence.

To define a more detailed risk scenario it is then necessary to account for possible sources of contamination, the exposed receptors (human and ecological) and the environmental levels of the added pollutants. The addition of new pollutants may better describe the environmental analysis but requires that data on their relative physico-chemical and toxicological properties are defined. These data can be derived from peer-reviewed literature, international databases, experimental values or predicted using quantitative structure–activity relationship (QSAR) models.

Whenever a pollutant is fundamental to define the environmental quality but its profile is incomplete, the inclusion can be done in a conservative way using:

- The maximum score for the lacking physico-chemicals properties (e.g., solubility, persistence, BCF, environmental distribution);
- The risk threshold in the case of undefined reference dose (PNEC, RfD or slope factor).

However, it is advisable to avoid the inclusion of a compound with an incomplete set of information to prevent boost of uncertainty.

2.1.4 Ecotoxicological and toxicological values (Peer-reviewed literature and databases, slope factors, reference dose, EC50, NOAEL)

We used Predicted No-Effect Concentration ecotoxicological values (PNECs), physico-chemical properties and environmental fate parameters from peer-reviewed databases like ECOTOX (USEPA, 2007), TOXNET (HSDB, 2009), INERIS (INERIS, 2009), RAIS (RAIS, 2009), Chem ID Plus (Chem ID Plus, 2009), RTECS® (CCOHS, 2009), HazDat (ATSDR, 2001) and specific reviews for some critical compounds.

If different ecotoxicological data exist for the same compound, values were selected by applying the rules in the Risk Assessment Technical Guidance (European Community, 2003), updated within the REACH legislation in 2006 (European Community, 2006). These rules are summarized here with other criteria from peer-reviewed documents:

- most sensitive species;
- typical standard tests are preferred as reported in the guidelines
(e.g. OECD 305 test for bioconcentration factor (OECD, 1996);
- peer-reviewed and official papers.

The applied safety factors are: median effect or lethal concentration (EC50 or LC50) divided by 1000 in case of data of acute toxicity (short-term, e.g. 4 days for fish), by 100 for sub-acute toxicity data (No Observed Effect Level, medium term, e.g. 21 days for fish) and divided by 10 for sub-acute toxicity data (Chronic = long term, e.g. 30 days for fish).

The Human Quality Index (HQ) and Cancer Risk Index (CR) are calculated using the toxic and carcinogenic parameters for human risk assessment (e.g. Reference Dose, Slope Factor, Chronic Daily Intake) from updated, reliable guidelines such as the Risk assessment Guidance for Superfund (USEPA, 1989), Environmental and Human Italian Protection Agency (APAT, 2008) and Guidelines for Carcinogen Risk Assessment (USEPA, 2005). Toxicological values for the selected pollutants were obtained from ISS/ISPESL and IRIS databases (ISS/ISPESL, 2009; USEPA, 2009a).

2.1.5 Predictive software and modelling resources (QSAR predictions, Mackay model)

Freely available software were used for ERICA in case of missing or unreliable experimental data. The QSAR programs used to predict values were EPI Suite v. 4.0, ACD v. 10, DEMETRA and CAESAR. The latest versions of these models were used to calculate missing indicators such as solubility, LogP (logarithm of the octanol–water partition coefficient) and Koc (soil adsorption coefficient) as listed in Table 2.2.

Table 2.2 Overview of the software applied and relative endpoints

Software	Environmental, air and soil endpoints ^{1,2,3}	Physico chemical properties ⁴	Fate Parameters ⁵	Carcinogenicity ⁶	Mutagenicity ⁷
DRAGON					
DEMETRA					
EPISuite					
CAESAR					
ACD					

¹Environmental water endpoints: Fish LC50 96 h, Fathead Minnow LC50, Daphnia EC50. ²Environmental soil endpoints: Bird Acute oral toxicity LD50 14-days exposure, rat oral LD50. ³Environmental air endpoints: probability inhalation acute toxicity on rat, probability inhalation acute toxicity on mouse. ⁴Physico-chemical properties: MW, Log KOA, Log Kow, water solubility, melting point, boiling point, and vapour pressure of organic chemicals. ⁵Fate Parameters: BCF, BAF, Henry's Law constant (air/water partition coefficient), aerobic and anaerobic biodegradability of organic chemicals, level III multimedia fugacity model (predicts partitioning of chemicals among air, soil, sediment, and water under steady state conditions), biodegradation half-life for compounds containing only carbon and hydrogen, aerobic and anaerobic biodegradability of organic chemicals, aerobic biodegradability. ⁶Carcinogenicity: classification models, probability female/male mouse, probability female/male rat, weight of evidence carcinogenicity. ⁷ Mutagenicity: classification models, Ames mutagenicity

The predictive abilities of the models selected for populating the ERICA database were evaluated as described in **CHAPTER 3**. For each model, particular attention was paid to the evaluation of the applicability domain, transparency and model reproducibility (Eriksson et al., 2003). For example, in case of missing data the predicted acute toxicity for rainbow trout (*Oncorhynchus mykiss*) was calculated with the DEMETRA free and validated models. DEMETRA models evaluate the ecotoxicity of pesticides addressing the Directive 91/414 on pesticides, but they are also appropriate for other environmental pollutants (Benfenati, 2007; Benfenati et al., 2007).

We also used CAESAR QSAR models, specifically built for use under REACH legislation, in case of missing or unreliable data for the following endpoints: bioconcentration factor, mutagenicity, and carcinogenicity.

Fish model for aquatic toxicity and bioconcentration factor BCF model are treated in details in **CHAPTER 3** together with the uncertainty due to values coming from experimental and predictive methods.

To calculate the time scale for distribution of the pollutant in the environmental compartment we used the Level III Fugacity Model EPI Suite (Level III Mackay) (USEPA, 2010) with the environmental parameters described in Mackay et al. (1992) as default values. We selected this model for its ability to predict the partitioning of an organic compound in a representative environment (Mackay et al., 1996). The Level III model in EPI Suite assumes steady state but not equilibrium conditions and allows predictions for partitioning between air, soil, sediment and water using a combination of default parameters and various input parameters that may be defined or estimated by other programs within EPI Suite (USEPA, 2010).

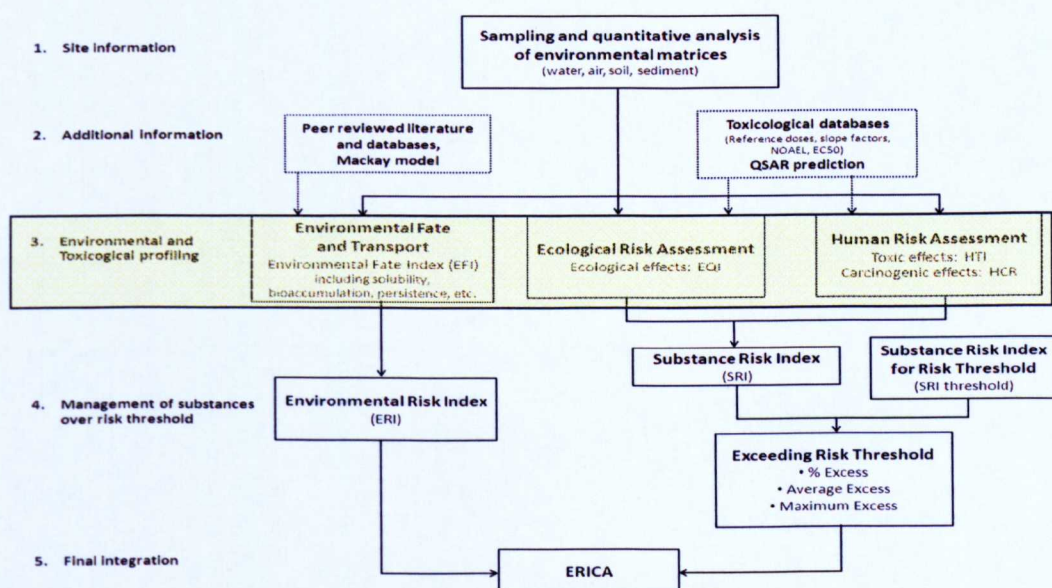
2.2 Scoring systems

The scoring system is an important point in the structure of ERICA because it allows the management and the integration of the proposed parameters characterized by different units of measurements. In the Index, the use of an objective scoring system is also useful to include the environmental properties (mobility, persistence, water solubility, volatility and bioaccumulation tendency) of selected compounds into a single parameter (EFcompound) used to describe the environmental fate of the pollutants.

All the adopted scoring systems for ecotoxicological, toxicological and physico-chemical parameters are reported in **Appendix 2**.

All the files containing the ERICA database, the Excel sheet with the calculation are in **Appendix 1**.

2.2 Theory and calculation (part 3 fig 2.1)



2.3.1. Ecotoxicological risk assessment

ERICA is a tiered index for environmental risk evaluation based on a triad approach including ecotoxicological risk evaluation, human risk assessment and environmental fate and transport (see Fig. 2.1).

These three main components are integrated into a single value using a dedicated scoring system that takes into account the different physico-chemical properties and toxicological profiles of the toxicants. The physico-chemical properties are included in the environmental fate and transport component together with the criteria to define a substance as Persistent, Bioaccumulative and Toxic (PBT) derived from the recent guidelines of the Environmental Chemical Agency (ECHA, 2008). The toxicological information is integrated in ecotoxicological or human risk assessment indices considering the risk threshold and quantifying the numbers and the extent of values in excess.

Below I show how the ERICA index is obtained. Briefly, the final equations (Eq. (21)) identify the amount of threshold exceeded and the possible impact on human and ecological healthiness.

For this purpose an index is defined to quantify the global exceedance values (ERIE, Eq. (19)), based on the indices for the health effect (SRI, Eq. (8)) and for environmental behaviour (EFI, Eq. (13)) of each pollutant.

The components of ecotoxicological risk assessment used in ERICA include the traditional risk procedures and the environmental distribution of the selected toxicants.

Results from chemical analysis of the environmental matrices are used to calculate the Ecological Quality index (EQ).

This index determines for each chemical if its environmental concentration is higher or lower than a level which may pose a risk as PEC values. Thus, EQ is calculated as the ratio PEC/PNEC as in (Eq. (1))

$$\text{Eq. (1)} \quad \text{EQ} = \text{PEC/PNEC}$$

where

PEC= Predicted Environmental Concentration for a selected compound;

PNEC = Predicted No-Effect Concentration for a selected compound.

When $\text{EQ} \geq 1$ there is a possible risk. Results from EQ are rated using a scoring system inspired by Finizio et al. (2001) [in **Appendix 2** are reported all the references to the scoring system].

EQ values are calculated for each environmental matrix (air, soil, sediment and water) and translated in the relative dedicated scores (see **Appendix 2**) which are toxic categories derived from the EQ values. The scores are integrated with the information about environmental distribution in the environmental matrices above defined (D) for the compound of interest (see below).

The Integrated index, called Ecotoxicological Quality Index (EQI) (Eq. (2)), is obtained using the following formula:

$$\text{Eq. (2)} \quad \text{EQI} = (\text{sEQ}_{\text{soil}} \times \text{D}_{\text{soil}}) + (\text{sEQ}_{\text{water+sediment}} \times \text{D}_{\text{water+sediment}}) + (\text{sEQ}_{\text{air}} \times \text{D}_{\text{air}})$$

where

EQI = Ecotoxicological Quality Index [dimensionless] (see **Appendix 2**);

D_{soil, water or air} = score for distribution of the compound into environmental compartments (calculated using Level III Fugacity Model – EPI SUITE v. 4.00);

sEQ_{soil, water or air} = score for environmental effects due to the toxicant in soil, water, sediment or air. sEQ range is 0.5 - 32 (see **Appendix 2**).

D takes into account the percentage distribution of the compound derived from the fugacity model, using the following equation (Eq. (3)):

$$\text{Eq. (3) } D = 1 + [(9.5 * \text{distribution \%}) / 100]$$

where

D = environmental distribution of toxicant [dimensionless], following Mackay model from EpiWeb 4. D range is 1 – 10.5;

9.5 = adjustment factor to relate the score;

Distribution % = percentage of distribution of the toxicant in the selected matrix.

2.3.2 Risk Assessment for Human Health

The human risk assessment comprises two modules investigating toxic but non-carcinogenic and carcinogenic effects. Data on pollutant levels in the

environmental matrices and their distribution in the main compartments are used to calculate the Chronic Daily Intake (CDI) of toxicants due to exposure of the human target receptors to the environmental matrix. CDI can be calculated with specific risk assessment software or using the procedures described in international guidelines (see **Appendix 2**).

The ERICA input parameters for the human target must be set on a residential child [see **Appendix 2**, eq. A – E]. This was considered as the most conservative scenario and it gives a sensitive output for human risk assessment.

Other settings can be based on international guidelines, if the user wants to apply ERICA for different scenario such as occupational assessment.

CDI is then used to calculate the Human Quality Index (HQ) and the Human Cancer Risk (CR), the two components of the human risk assessment index.

The Human Quality Index (HQ) (Eq.4) is the part used to quantify the possible toxic effects on human receptors. It compares the calculated CDI with an estimated daily oral exposure of the human population (including sensitive subgroups) without an appreciable risk of adverse effects during a lifetime. The HQ can be calculated with the formula:

$$\text{Eq. (4)} \quad \text{HQ} = \text{CDI}/\text{RfD}$$

where

HQ = estimated toxic effects of the substance [dimensionless];

CDI = chronic daily intake [mg (kg d)⁻¹];

RfD = reference dose [mg (kg d)⁻¹].

Reference dose is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a NOAEL, LOAEL, or benchmark dose, with uncertainty factors generally applied to reflect limitations of the data used. Generally it is used in non-cancer health assessments. Durations include acute, short-term, subchronic, and chronic (USEPA, 1993).

Benchmark dose is a dose or concentration that produces a predetermined change in response rate of an adverse effect (called the benchmark response or BMR) compared to background.

NOAEL is the highest dose or exposure level at which a statistically or biologically significant effect is not observed in the exposed population compared with an appropriate unexposed control group.

LOAEL is the lowest exposure level at which there are level at which there is biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control group.

This relationship indicates how much the exposure from the environment exceeds the tolerable dose, and is conceptually related to the EQ (Eq 1).

The CR (Eq. 5), instead, describes the increase in tumor probability due to exposure to carcinogenic substances in habitual living conditions. The estimated carcinogenic effect is calculated by multiplying the CDI by the cancer risk associated with the dose of a carcinogen (slope factor), using the following equation:

Eq. (5)

$$CR = CDI \times SF$$

where

CR = estimated carcinogenic effect of the toxicant [dimensionless];

CDI = Chronic Daily Intake [mg (kg d)⁻¹];

SF = slope factor [mg⁻¹ kg d].

The slope factor is the cancer risk per unit of dose. It is calculated as the highest estimated probability of an individual developing cancer if exposed to a chemical by ingestion for a lifetime of 70 years, approximating a 95% confidence limit.

Since risk at low exposure levels cannot be measured directly either by animal experiments or by epidemiologic studies, a number of mathematical models and procedures have been developed for this use. Different extrapolation models or procedures, while they may reasonably fit the observed data, may lead to large differences in the projected risk at low doses. When data are limited, however, and when uncertainty exists regarding the mechanisms of carcinogenic action, models or procedures which incorporate low-dose linearity are preferred when compatible with the information available. For example EPA usually employs the linearized multistage procedure in the absence of adequate information to the contrary. As explained in paragraph 2.1.2 the slope factors used in ERICA were obtained from selected databases such as IRIS (see 2.1.4).

Results about toxic and carcinogenic effects are translated into comparable values using a dedicated scoring system (see **Appendix 2**, Tab. 2 and 3), then integrated respectively, in the Human Toxicological Index (HTI) and in the Human Cancer Risk (HCR). These two parameters take into account the adverse effects on target receptors together with the environmental distribution of the selected compound.

HTI (*Eq. 6*) and HCR (*Eq. 7*) are obtained applying these equations:

Eq. (6) $HTI = (sHQ_{soil} \times D_{soil}) + (sHQ_{water+sediment} \times D_{water+sediment}) + (sHQ_{air} \times D_{air})$

Eq. (7) $HCR = (sCR_{soil} \times D_{soil}) + (sCR_{water+sediment} \times D_{water+sediment}) + (sCR_{air} \times D_{air})$

where

HTI = human toxicological index to estimate the toxic effects on human receptors [dimensionless];

HCR = human cancer risk for estimating of carcinogenic effects in human populations [dimensionless];

sHQ soil, water or air = score for human toxic effects due to the toxicant in soil, water, sediment or air [dimensionless] (see Appendix 2);

sCR soil, water or air = score for human carcinogenic effects due to the toxicant in soil, water, sediment or air [dimensionless] (see Appendix 2);

D = environmental distribution of toxicant [dimensionless]

2.3.3 INTEGRATION OF HUMAN AND ECOLOGICAL ASSESSMENT

Human risk assessment (HTI and HCR) and ecotoxicological risk assessment (EQI) must be carried out for each pollutant and the results are combined to define the Substance Risk Index (SRI), used to describe the overall effects of a compound on human populations and ecological organisms (plants and animals). HTI and HCR equations are implemented in the Excel file with all the calculations to obtain ERICA (Appendix 1). To define the SRI we assigned an equal “weight”

to the effects on ecological and human targets (see Fig. 2.1), in order to account for the strict relationship between human health and environmental quality. In this way the evaluation of pollutant impact on both kinds of receptors is balanced: a multiplying factor of 0.25 was assigned to each part of the human risk assessment (HTI and HCR) in order to equally counterbalance the weight (0.5) of ecotoxicological risk assessment. So SRI is defined by the formula (Eq. 8):

$$\text{Eq. (8)} \quad \text{SRI} = (0.5 \times \text{EQI}) + (0.25 \times \text{HTI}) + (0.25 \times \text{HCR})$$

The SRI is one of the components of the Environmental Risk Index (ERI) and quantifies the adverse effects on receptors, considering also the environmental fate of the toxicant (D value, see Eq 7).

ERI (Eq. 9) is obtained using the formula:

$$\text{Eq. (9)} \quad \text{ERI} = \text{SRI} \times \text{EFI}$$

Where

ERI = environmental risk index [dimensionless];

EFI = environmental fate index [dimensionless], describing the environmental fate and transport of the toxicants.

The EFI indicates the potential danger of exposure on a time scale. It is based on the fate and environmental properties (mobility, persistence, water solubility, volatility and bioaccumulation tendency). This measure shows if the levels of a compound could rise over time, becoming a matter of concern for the future. EFI is built to be a parameter to prioritize compounds of an ERICA scenario. It introduces an additional risk related to the danger of the persistency and bioaccumulation of the compound in the environment over time. More information

is available regarding the chemical (degradation, biodegradation, composition of the soil, degree of emission in the environment, future plans for the site under study, etc.). The behaviour of the compound in the environment over time can be described in a realistic way. The EFI relationship (Eq. 12) is quantified in the following equation:

$$\text{Eq. (10) } \text{EFI} = 1 + [(\text{EF}_{\text{compound}} - \text{EF}_{\text{min}}) / \text{EF}_{\text{max}}]$$

where

$\text{EF}_{\text{compound}}$ = environmental fate of the compound [unitless] (eq. 13) (see below and Appendix 2);

EF_{min} = minimum EF (= 2.67); (see Appendix 2);

EF_{max} = maximum EF (= 25) (see Appendix 2);

The Environmental Fate of the toxicant ($\text{EF}_{\text{compound}}$) is related to the physico-chemical properties of the pollutants (Eq. 13). It quantifies the most important properties influencing the behaviour of the xenobiotic in the environmental matrix. The formula to calculate EF of a compound is the following:

$$\text{Eq. (11) } \text{EF}_{\text{compound}} = (\text{S} + \text{M}) / \text{V} + \text{BCF} + \text{P}$$

Where:

S = score for the water solubility of the compound [see Appendix 2, Tab. 4];

M = score for the mobility of the compound, based on Koc value [see Appendix 2, Tab. 5];

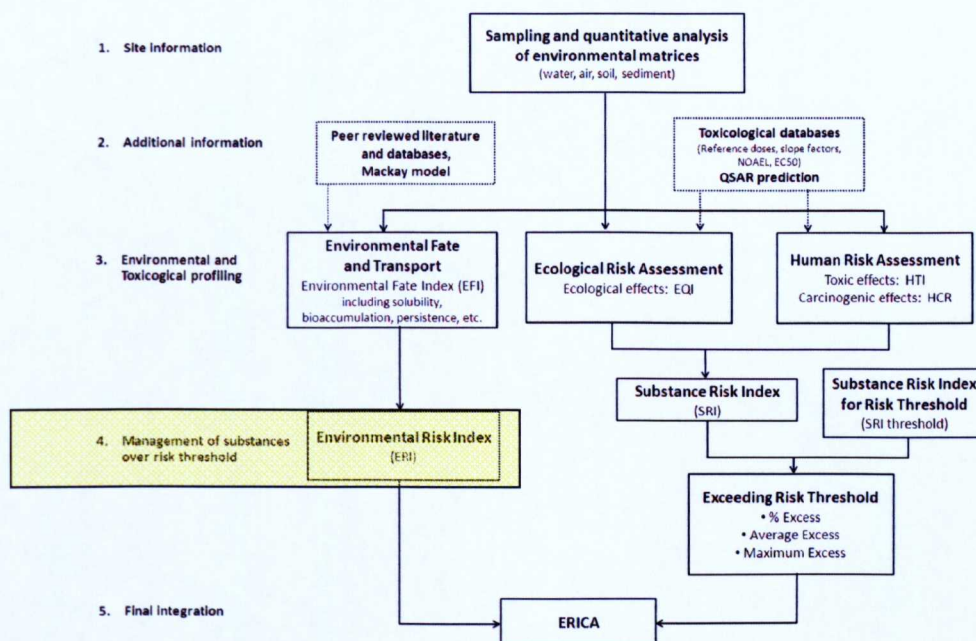
V = score for the volatility of the pollutants, based on its vapour pressure [see Appendix 2, Tab. 6];

BCF = score for the bioconcentration property, expressed as the logarithm of compound's BCF or BAF [see Appendix 2, Tab. 7];

P = score for the persistence of the pollutant, described as degradation time [see Appendix 2, Tab. 8].

Other physico-chemical properties might be added to the definition of the EF subindex following future developments and data availability. For example, data on photolysis could be useful to describe the stability of a pollutant when it is released into an environmental matrix.

2.3.4 MANAGING TOXICANTS EXCEEDING THE RISK THRESHOLD



An increased probability of adverse effect on human and environmental targets is linked to the exceeding of the risk threshold by a substance.

For each priority pollutant, the SRI is compared with the *risk threshold* to verify if a pollutant's effect exceeds the safety level. The Pollutant Risk Index, PI (*Eq. 12*) is described by the equation:

$$\text{Eq. (12) } PI = \text{SRI}_{\text{toxicant}} / \text{SRI}_{\text{threshold}}$$

The number of toxicants with pollutant risk >1 must be noted down as number of pollutants exceeding the risk threshold, (number of exceeding pollutants, NEP) and it will be successively used to calculate the percentage of toxicants that

exceed risk threshold (Eq. 14). For each exceeding value, the Exceeding Risk (ER) (Eq. 13) is calculated as follow:

$$\text{Eq. (13)} \quad \text{ER} = \text{Pollutant Risk} - 1$$

We decided to enhance this possible hazard by introducing a set of additional parameters for pollutants that exceed the risk threshold (as described by Eq. 12 and Eq. 13). We may have cases where there are many of them, or cases where there is a single pollutant with a high value. These two situations have to be considered. First of all, NEP is used to obtain their percentage by the formula:

$$\text{Eq. (14)} \quad \%E = (\text{NEP} / \text{NIC}) \times 100$$

where

$\%E$ = percentage of toxicants that exceed the risk threshold [%];

NEP = number of pollutants exceeding the risk threshold [dimensionless];

NIC = number of investigated priority compounds [dimensionless].

Then, the average and the maximum of ER, average exceeding (AE), and the maximum exceeding (ME), are calculated. These last two factors are used to obtain the Integrated Threshold Exceeding (ITE) using the formula:

$$\text{Eq. (15)} \quad \text{ITE} = 1 + (\text{AE} \times \text{ME})$$

2.3.5 ERICA FOR MACROPOLLUTANTS

A large number of studies have been done on the toxicity of macropollutants such as ozone, particulate matter (PM₁₀ and PM_{2.5}), carbon monoxide, nitrogen dioxide and sulphur dioxide. In 1999, USEPA released the Air Quality Index (AQI), a simplified method to evaluate the probability of adverse effects in human and environmental targets exposed to this group of substances (USEPA, 1999). The AQI is a daily index for reporting air quality and focuses on health and environmental effects due to exposure to polluted air. U.S. EPA calculates the AQI for the six major air pollutants regulated by the Clean Air Act. Under this act, EPA has established National Ambient Air Quality Standards (NAAQS) that can be used as reference doses to protect public health (Primary Standards) and the environment (Secondary Standard). These values are periodically revised on the basis of epidemiological studies.

On the basis of this higher data availability, we created the specific approach for macropollutants inspiring by AQI approach because it is scientifically based but easily understandable. We used different methods to calculate AQI for the assessment of human health (*Eq. 16*) and for the environment (*Eq. 17*).

The AQI equation for human targets is

$$\text{Eq. (16) } AQI = [(IHI - ILO) / (BPCHI - BPCLO)] \times (C - BPCLO) + ILO$$

where

AQI = Air Quality Index for the selected pollutant [dimensionless];

IHI = AQI values corresponding to BPCHI [dimensionless];

ILO = AQI values corresponding to BPCLO [dimensionless];

BPCHi = breakpoint concentration equal or greater than C for the selected pollutant [mg m⁻³];

BPCLo = breakpoint concentration less than C for the selected compound [mg m⁻³];

C = concentration of the selected pollutant [mg m⁻³].

A breakpoint concentration is the maximum concentration of the chemical before a hazard occurs for environment and populations. This value is based on first and secondary NAAQS and is reported in AQI technical guidance (USEPA, 2009) and in Appendix 2.

For environmental risk assessment the AQI formula is:

Eq. (17) $AQI = (100 \times C) / NAAQS$

Where:

AQI = Air Quality Index for the selected pollutant [dimensionless];

C = concentration of the selected pollutant [mg m⁻³];

NAAQS = secondary standard for investigated compound [mg m⁻³].

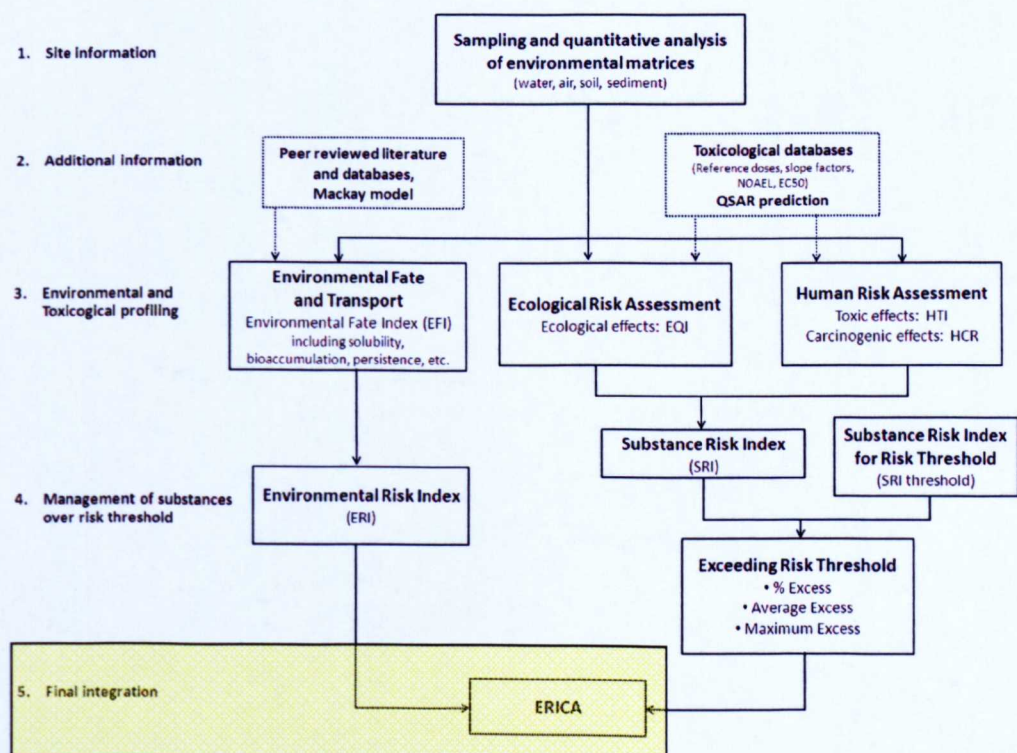
An AQI value of 100 corresponds to the NAAQS for the pollutant, which is the safety level set by EPA to protect public health and the environment. AQI values below 100 are satisfactory but when they are above 100 the air quality is

AQI is fully referenced and transparent and it was added to ERICA for macroinorganic toxicants (see **Appendix 2**, Tab. 9) using a dedicated scoring system. AQI values are directly integrated into ERI using the following equation:

Where:

sAQIeco= score corresponding to the AQI data for the ecological target;

2.3.6 FINAL INTEGRATIONS



The ERI must be calculated for each priority pollutant and the results are analyzed to obtain the average ERI for the investigated scenario (Eq. 19):

Eq. (19) $\langle \text{ERI} \rangle = (\text{ERI}_1 + \text{ERI}_2 + \text{ERI}_3 + \dots + \text{ERI}_X) / x$

ERI is an important parameter to describe the global situation of an investigated area and it is integrated with the previously defined number of pollutants exceeding the risk threshold. The integrated parameter is called the Environmental Risk Index and Exceeding value (ERIE) and takes into account the percentage of toxicants above the risk threshold and the average ERI values for the investigated scenario.

ERIE is obtained as follows:

Eq. (20)
$$\text{ERIE} = (1 + E\%) \times \langle \text{ERI} \rangle$$

Where:

ERIE = Environmental Risk Index and Exceeding value [dimensionless];

%E = percentage of toxicants that exceed risk threshold (see Eq. 14).

Finally, ERICA can be calculated. This final index integrates data from human and ecotoxicological risk assessment, physico-chemical based environmental fate and data on risk threshold excesses. The equation used to derive ERICA is:

Eq. (21)
$$\text{ERICA} = [(\text{ERIE} \times 100) / \text{ERIE risk threshold}] \times \text{ITE}$$

Where:

ERICA = environmental risk index for a complete assessment [unitless];

ERIE risk threshold = ERIE value corresponding to risk threshold [unitless];

ITE = Integrate Threshold Exceeding (see Eq. 15).

The ERICA final value is used to define the Environmental Quality by a tiered classification similar to the “Air Quality Index” (USEPA, 1999). It is divided into eight categories from “very good” to “hazardous”. Each category corresponds to a different level of environmental health concern.

The eight levels (see Fig. 2.2) are:

- “Very good” ERICA is 0 - <25, the environmental health quality is satisfactory and pollution poses no risk for human and ecological receptors;
- “Good” ERICA is 25 – 49, the environmental health quality is satisfactory and pollution causes little risk;
- “Moderate” ERICA is 50 – 99 the environmental health quality is acceptable but there may be a moderate health concern for some pollutants;
- “Unhealthy for sensitive groups” ERICA is 100 – 149, human and ecological targets are not affected by risk but the most sensitive receptors (e.g. people with heart and lung disease, children and older adults) start to be affected by risk;
- “Unhealthy” ERICA is 150 – 199, every target may begin to experience some adverse effects and most sensitive ones may be subject to risk;
- “Very Unhealthy” ERICA is 200 – 299; this category could trigger health or environmental alert because all receptors could be affected by risks;
- “Dangerous” ERICA is 300 – 399, and targets are in danger with substantial risks;
- “Extremely dangerous” ERICA is > 400, corresponding to emergency conditions because all the receptors are affected by serious adverse risks.

Following the USEPA approach, a colour was selected for each ERICA category to make the results easy to understand for the audience.

Fig 2.2: The eight levels of concern describing the health status of a territory with ERICA.

Environmental and health status	
Environmental Quality	ERICA
VERY GOOD	< 25
GOOD	25-49
MODERATE	50-99
UNHEALTHY FOR SENSITIVE GROUPS	100-149
UNHEALTHY	150-199
VERY UNHEALTHY	200-299
DANGEROUS	300-399
EXTREMELY DANGEROUS	> 400

CHAPTER 3: Evaluation of methods to select experimental and predictive values for the properties of the compounds including uncertainty parameters.

3.1 Introduction

The main sources of data used to create the ERICA database were presented briefly in CHAPTER 1; in this chapter the work done to validate the models used to predict the missing experimental or unreliable values is described. The uncertainty related to the experimental data and the predictive models is also treated.

Both the topics of validation of predictive methods and uncertainty related to the process of data source are at present the subject of debate in the scientific world, because of new legislations for industrial products like the REACH Directive (REACH, 2006) or the Cosmetic Directive (Commission Directive 2008/42/EC of 3 April 2008). Both directives aim to reduce the impact of chemicals on human health and the environment, to reduce the number of experiments using alternative testing strategies and to promote risk assessment analysis for evaluation of the health status of the environment.

Due to the present lack of data on chemicals in Europe and to the limitations of assays, REACH supports full use of all types of data (*in vivo*, *in vitro*, *in silico*), while the Cosmetic legislation aims to use the 3 R principles. 3R stands for: Replacement of animal experiments, Reduction of animal experiments and Refinement of experiments –the latter meaning the improvement of methods to minimize pain of experimental animals.

Nowadays it is necessary to assess how heterogeneous data coming from different testing strategies can be managed within a unified approach suitable for

the risk characterization of the chemicals, reducing all the typologies of uncertainty related to different steps.

The paragraphs in this chapter present various sources of uncertainties:

3.2) an example on variability for experimental data (bioconcentration endpoint),

3.3) two examples of work done comparing predictive tools:

A) DEMETRA and B) CAESAR models, on the results for two different ecotoxicological endpoints.

3.2 Uncertainty in experimental data: example with the analysis of experimental data, variability for the indicator bioconcentration factor (BCF)

Experimental data variability was analysed for various indicators which were part of the index. In particular, the endpoint bioconcentration factor (BCF) was analysed in detail because it gave central information on assessing the ecotoxicological risk and accumulation of a compound in the dietary chain.

3.2.1 Bioconcentration factor (BCF)

According to international guidelines “bioaccumulation” is defined as the process where the chemical concentration in an aquatic organism achieves a level that exceeds that in the water as a result of chemical uptake through all routes of chemical exposure (e.g. dietary absorption, transport across the respiratory surface, dermal absorption). Bioaccumulation typically takes place under field conditions and is a combination of chemical bioconcentration and biomagnification (the process by which lipid normalized chemical concentrations increase with trophic level in a food-chain).

The extent of chemical bioaccumulation is usually expressed in the form of a bioaccumulation factor (BAF), which is the ratio of the chemical concentration in the organism (CB) and the water (CW), including the uptake in the diet.

Bioconcentration is the process where the chemical concentration in an aquatic organism achieves a level that exceeds that in the water as a result of the exposure of an organism to a chemical in the water but does not include exposure via the diet. Bioconcentration refers to a situation, typically derived under controlled laboratory conditions, where the chemical is absorbed from the water via the respiratory surface and/or the skin only. The extent of chemical bioconcentration is usually expressed in the form of a bioconcentration factor (BCF).

BCF is the concentration of test substance in or on the fish or specified tissues divided by the concentration of the chemical in the surrounding medium at steady state. In the context of setting exposure criteria it is generally understood that the terms "BCF" and "steady-state BCF" are synonymous. A steady-state condition occurs when the organism is exposed for a sufficient length of time sufficient for the ratio not to change substantially.

BCFs are used to relate pollutant residues in aquatic organisms to the pollutant concentration in ambient waters. Many chemical compounds, especially those with a hydrophobic component, partition easily into the lipids and lipid membranes of organisms and bioaccumulate.

BCF and BAF are described by the following equations:

$$\text{Eq(22) } \text{BCF} = \text{CB/CWD} = k_1 / (k_2 + k_E + k_M + k_G)$$

$$\text{Eq (23) } \text{BAF} = \text{CB/CWD} = \{k_1 + k_D (\text{CB/CWD})\} / (k_2 + k_E + k_M + k_G)$$

Where CB is the chemical concentration in the organism (g/kg-1), k_1 is the chemical uptake rate constant from the water at the respiratory surface ($\text{L}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$), CWD is the freely dissolved chemical concentration in the water ($\text{g}\cdot\text{L}^{-1}$), k_D is the uptake rate constant for chemical in the diet ($\text{kg}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$) and k_2 , k_E , k_M , k_G are rate constants (d^{-1}) representing chemical elimination from

the organism via the respiratory surface, fecal egestion, metabolic biotransformation, and growth dilution, respectively.

3.2.2 Recent use of BCF

In particular BCF is a very valuable endpoint now used mainly for:

- **Classification & Labelling (C&L):** All substances should be assessed for environmental hazard classification. Bioaccumulation potential is one aspect that needs to be considered in relation to long-term effects.
- **Prioritization (PBT – persistency - bioconcentration – toxicity; vPvB – very persistent – very bioaccumulative):** bioaccumulation is one of the criteria used for the PBT/vPvB assessment. For a definitive conclusion, reliable measured BCF data are generally necessary (for fish or an invertebrate such as mollusc). However, a provisional assessment can be made against screening criteria. To define if a chemical is PBT or vPvB the thresholds for REACH in Europe are: for B $BCF > 2000 \text{ L/kg}$ (whole organism weight) = 3.3 in Log unit vB $BCF > 5000 \text{ L/kg}$ = 3.7 in Log unit
- **Chemical Safety Assessment (CSA):** fish BCF and BMF (Biomagnification Factor) values are used to calculate concentrations in fish as part of the secondary poisoning assessment for wildlife, as well as for human dietary exposure. An invertebrate BCF may also be used to model a food chain based on consumption of sediment worms or shellfish. An assessment of secondary poisoning or human exposure via the environment will not always be necessary for every substance. In the first instance, a predicted BCF may be used for first tier risk assessment.

The preferred experimental conditions for BCF test (as for example the one requested now under REACH legislation) are those reported in the OECD 305 guideline, where bioaccumulation is mentioned for the aquatic species, preferably fish. The likely number of fish recommended for this test is in the range 132 to

240, for a duration of 44-116 days; this results in a huge cost for each experiment, estimated in the range of 50-100 k€.

3.2.3 Experimental variability for BCF

The variability of the BCF data reported in the literature five years ago is ± 0.75 log units (Dimitrov et al. 2005). The variability of the experimental data (calculated as the average of the range assumed by the values for each compound) in the NRC CANADA database (Arnot and Gobas, 2006) is 0.69 log units. Considering only experimental data for fish species suggested by the OECD (according to OECD guideline 305) and with an overall reliability score of 1 (the most reliable data), the variability drops to 0.48 log units. For the EURAS database, considered a gold standard database, the variability of the experimental BCF values is 0.45 log units, which decreases to 0.42 log units for the substances included in the study reported in session 3.3 part B of this chapter.

Here I report analysis done to assess experimental variability of BCF values for the following databases:

3.2.4. NRC Canada database

3.2.5. EURAS database

3.2.4 NRC Canada database

The NRC Canada database (Arnot and Gobas, 2006) concerning BCF and BAF assessment for organic chemicals in aquatic organism was analysed focusing on logP and logBCF data.

The BCF endpoint was measured from total water concentrations.

Initially, all data concerning fish were taken into consideration. There was a high variability on the 759 chemicals: the maximum difference between the minimum and the maximum of logBCF was 6.10 and the higher value of standard deviation (sd) was 2.99.

The database is provided with a scoring system in which the overall score is obtained combining the confidence score (high, moderate or low) assigned at six confidence criteria (water analysis, radio-label, aqueous solubility, exposure duration, tissue analysis and other factors considered). The overall score is acceptable if the confidence score is high or moderate. In order to analyze the variability, the data relative to fish in which the overall score is 1 (acceptable confidence) were considered. Thus, the number of chemicals was brought down to 500 and the maximum difference between the minimum and the maximum of logBCF to 5.58, but the higher value of standard deviation was still steady.

Then the data relative only to the fish species recommended in the OECD 305 guideline were investigated. Considering only acceptable confidence data, the maximum difference between the minimum and the maximum of logBCF remain above 5 and the higher value of standard deviation was unchanged.

With the purpose of analyzing the interspecies difference, the data relative to *Oncorhynchus mychiss* (Rainbow trout) and *Cyprinus carpio* (Common carp) were separately evaluated. For both, there was no improvement in the difference between the minimum and the maximum of logBCF (about 5). There was a slight improvement for Rainbow trout in relation to the maximum of standard deviation (1,81 if only acceptable confidence data were considered).

Table 3.1: Analysis of data in NRC Canada database, number of compounds for different species, max standard deviation, % of compounds with sd > 0.3, max range (minimum value -maximum value)

Reliability of data	Number of chemicals	Max sd	sd > 0.3 (% of compounds)	Max range (min-max)
All fishes, all score	759	2.99	39.66	6.10
All fishes, score 1	500	2.99	34.60	5.58
OECD fishes, all score	693	2.99	28.57	5.86
OECD fishes, score 1	448	2.99	33.71	5.29
Common carp, all score	500	2.99	33.00	4.89
Common carp, score 1	313	3.04	25.56	4.89
Rainbow trout, all score	117	2.37	58.12	5.37
Rainbow trout, score 1	72	1.81	51.39	5.02

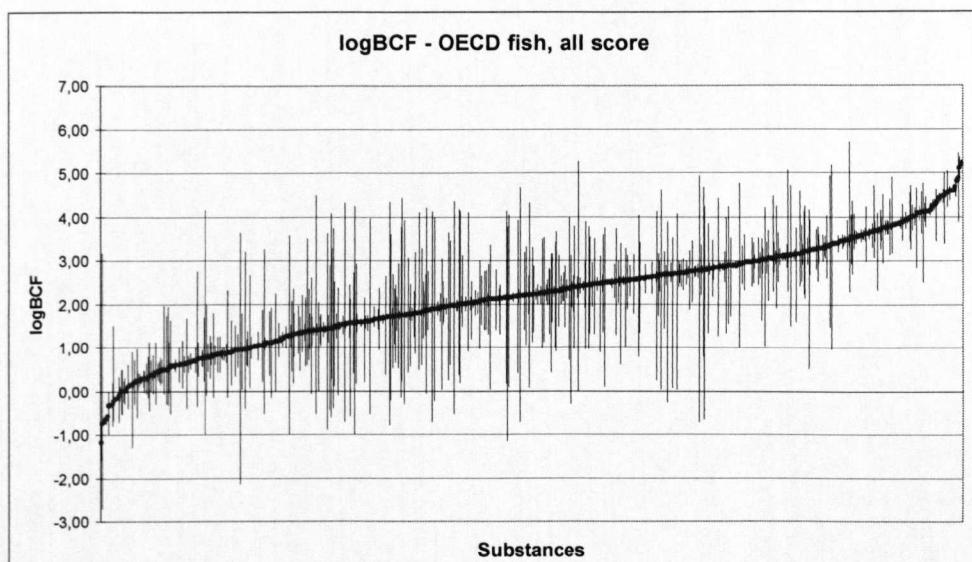
To evaluate the incidence of the method for measuring the BCF (both total water concentrations and freely dissolved concentrations), the chemicals which had logBCF measured with both methods were selected. There were no significant differences between the values obtained using different methodologies.

In conclusion, there is a high variability for the logBCF data. This variability is independent of the reliability of the data and of the fish species used in the test.

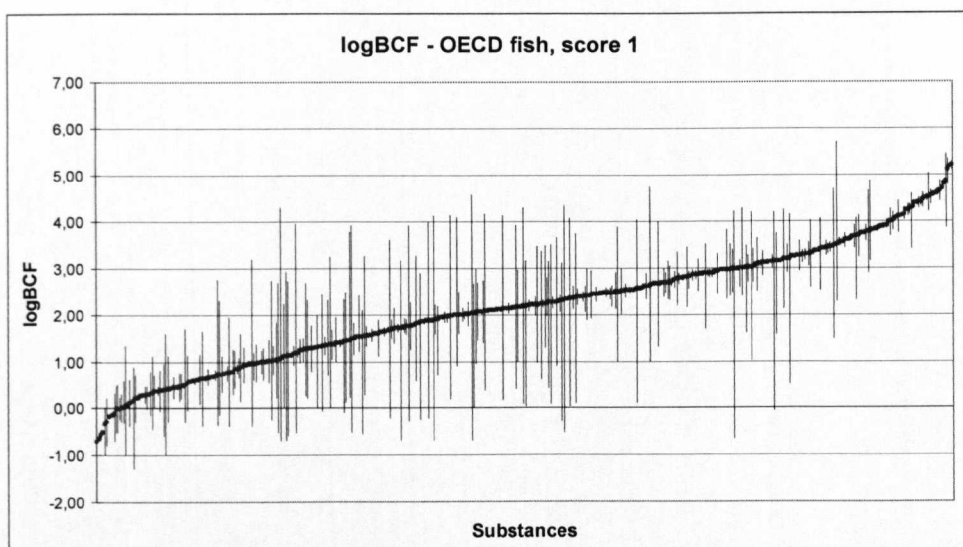
In figures 3.1a, 3.2b and 3.2c the minimum, maximum and mean values of BCF for each substance are reported for (a) the OECD fishes with all the acceptable scores, (b) the OECD fishes with only score 1 and (c) the Rainbow trout with only acceptable scores.

Fig 3.1: NRC Canada database: (a)variability of Log BCF for all the OECD fishes with all the acceptable scores, (b)variability of Log BCF for OECD fishes scored 1, (c)the Rainbow trout data with only score 1

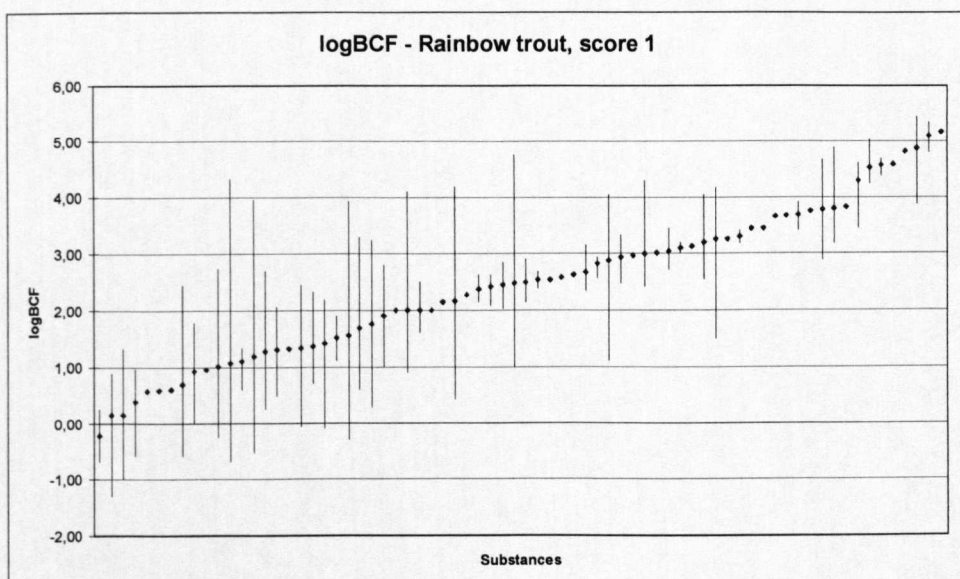
(a)



(b)



(c)



3.2.5 EURAS database

EURAS, the BCF “gold standard database” has been downloaded from the EURAS website. It contains 1130 records. To assess the variability of BCF data,

the EURAS database has been analyzed including multiple data for the same compounds.

The objective was to evaluate variability of data with respect to the reliability score and to assess the possibility of using the reliability score to quantify the quality of data.

First of all data with high reliability score (1 and 2) have been extracted from EURAS. Single values are not useful and have not been included.

Substances with multiple values and with reliability of 1 or 2 are 26, the number of data-points for the same compound range from 2 to 10.

This comparison highlights the extent of variability. As shown in table 3.2 and figures 3.2 data with high variability (1,2) have high difference value between minimum and maximum value. Data that are supposed to have the same quality have high differences in BCF value. One example is DDT, for which the database contains 10 BCF values, all with a reliability score of 2, with a maximum value of BCF = 89100 and a minimum value of BCF = 24 (sd=1.18).

For compounds which have no reliability score assigned, the same comparison has been done. 481 compounds have multiple values, mostly just 2 values.

In table 3.2 below the results are summarised:

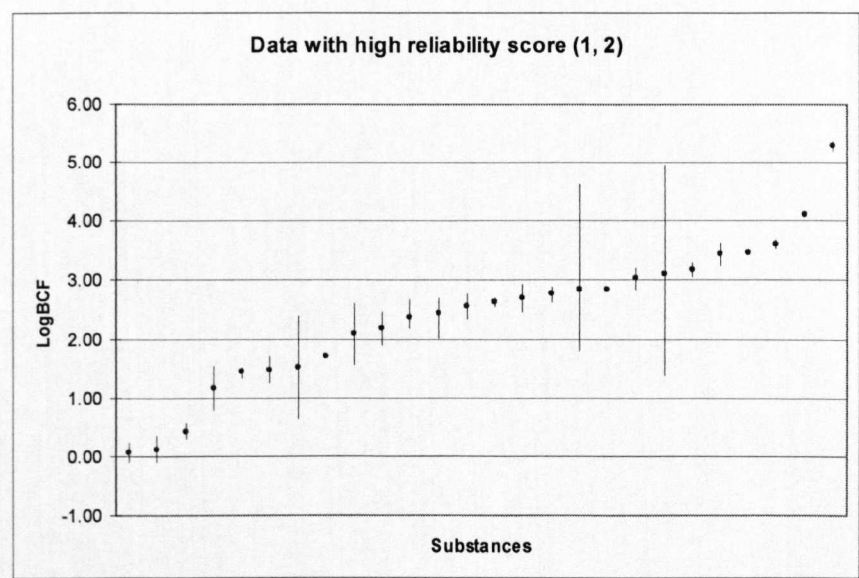
Table 3.2: Analysis of data in NRC Canada database, number of compounds for different species, max standard deviation, % of compounds with sd > 0.3, max range (minimum value -maximum value)

Reliability of data	Number of chemicals	Max sd	sd > 0.3 (% of compounds)	Max range (min-max)
High reliable (1, 2)	26	1.25	38.46	3.57
Without reliability score	481	1.18	38.25	1.67

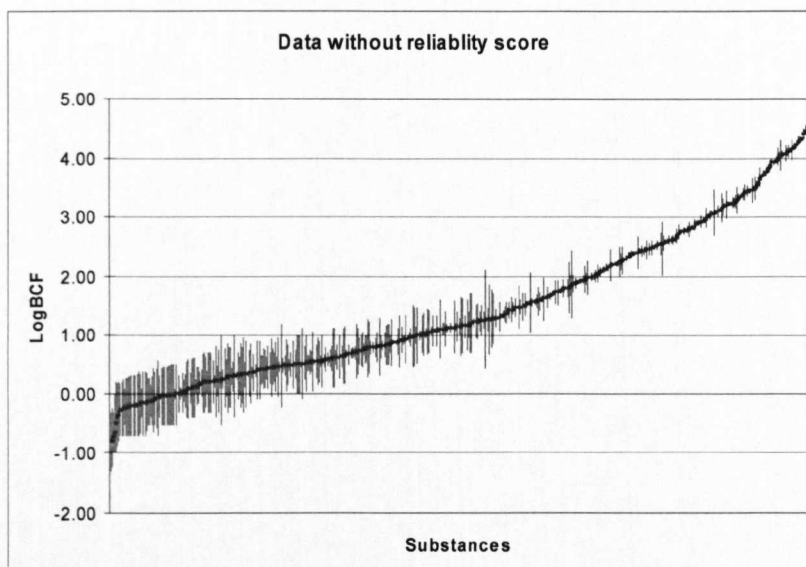
In the figures below the mean of LogBCF for each compound are reported and the distance between min and max value for (a) data highly reliable and (b) data without reliability score.

Fig 3.2 EURAS database: (a)Log BCF data with high reliability score, (b)Log BCF data without reliability score

(a)



(b)



3.3 Predictive methods and comparisons among predictive ability

3.3.1 Introduction

Quantitative Structure Activity Relationships (QSARs) are mathematical models that are used to predict measures of toxicity from physical characteristics of the structure of chemicals (known as molecular descriptors). Acute toxicities (such as the concentration that causes half of a fish population to die) are one example of the toxicity measures that may be predicted from QSARs. Simple QSAR models calculate the toxicity of chemicals using a simple linear function of molecular descriptors:

$$\text{Eq.(24) Toxicity} = ax_1 + bx_2 + c$$

where x_1 and x_2 are the independent descriptor variables and a , b , and c are fitted parameters. Examples of molecular descriptors include the molecular weight and the octanol-water partition coefficient. A very good guide about descriptors is the Molecular descriptors guide recently developed by USEPA (EPA v1.0.2, 2008).

Main uses of QSAR toxicity models

- QSAR toxicity predictions may be used to screen untested compounds in order to establish priorities for traditional bioassays, which are expensive and time-consuming.
- QSAR methodologies to estimate toxicity knowing only the molecular structure
- QSAR models are useful for estimating toxicities needed for green process (e.g. remediation, prioritization)

Transparency and model reproducibility are main factors to judge or compare SAR and QSAR model results. In particular, several statistical validation parameters (e.g. leave one out, external test set) and procedures to build the model (quality of data employed for the training set) give the proof that a model is robust. Furthermore, it is important that the applicability domain of the model is defined.

There are some restrictions in the use of the models. They are in general not suitable for:

- Inorganic compounds
- Mixture of chemicals
- Complexes.

The applicability domain of a model identifies the chemical classes outside of which the uncertainty of the model is higher. Among the parameters used to evaluate the predictive abilities of the models for ERICA, the applicability domain and the transparency of the method (equation available, explanation of the theory behind the model and statistical validation) were always checked.

To use in silico methods accurately it is necessary to know or measure their predictive ability and their inherent uncertainty and sensitivity. To evaluate the predictive abilities of a model there are different criteria depending on the specific application (quantitative – qualitative) of the model and from the type of information available for the endpoint.

Furthermore the data, their quality and number are at the basis of any QSAR model. Good quality data are very important to obtain a good QSAR model. Data quality is even more important in the case of read-across, which relies on very few values. Data quality is anyway at the basis of any assessment, in particular for regulatory purposes.

To assess properly the performance of a QSAR model it is important to know the specific variability of the endpoint of interest, as it will be implicitly transferred into the uncertainty of the QSAR model. A QSAR model cannot achieve predictions that are more accurate than the original data.

The main sources of SAR/QSAR modelling used in the index, as already reported in CHAPTER 1, Table 2 are the following software **EPI Suite v. 4.0**, **ACD v. 10**, **DEMETRA**, **CAESAR**, **SPARC**, while other software, free and commercial, were used to compare the predictive ability of the software used (e.g. TOPKAT®).

Indeed, an important additional parameter, which has to be evaluated, is if the error of the predictive alternative model is in one direction or in another, which means if the model produces more false positives or false negatives.

There are specific mathematical parameters to measure accuracy, specificity and sensitivity (Eq 25, Eq 26, Eq 27).

$$\text{Eq(25) } accuracy = \frac{TP + TN}{Tot}$$

$$\text{Eq(26) } sensitivity = \frac{TP}{TP + FN}$$

$$\text{Eq(27) } specificity = \frac{TN}{TN + FP}$$

where TP (true positives) is the number of correctly classified active compounds, TN (true negatives) is the number of correctly classified inactive compounds, FN (false negatives) is the number of misclassified active compounds and FP (false positives) is the number of misclassified inactive compounds.

It is possible to optimize the in silico model in one direction or in another. The decision varies depending on the strategy to be adopted, and in particular if the alternative method is included in an integrated testing strategy (ITS) or not. In the case of the use of a method within an ITS, it is preferable to evaluate the overall performances of the ITS, instead of the single method.

3.3.2 Free software:

- 1) **EPISUITE** <http://www.epa.gov/opptintr/exposure/pubs/episuite.htm>
- 2) **ECOSAR** <http://www.epa.gov/opptintr/exposure/pubs/episuite.htm>
- 3) **ACD** <http://www.acdlabs.com>
- 4) **DEMETERA** <http://www.demetra-tox.net>
- 5) **CAESAR** <http://www.caesar-project.eu>
- 6) **SPARC** <http://sparc.chem.uga.edu/sparc/>
- 7) **TOXTREE** <http://ecb.jrc.ec.europa.eu/qsar/qsar-tools/index.php?c=TOXTREE>

1) EPISUITE

EPISUITE contains many freely available models. The new version, EPIWEB 4.0, predicts physico-chemical properties like logP, gas phase reaction rate, Henry's constant, melting point, boiling point, vapour pressure, biodegradation, soil adsorption, and water solubility. For each model there is a fully referenced freely available manual that contains information about training tests (sometimes with experimental data for the diverse chemical classes), the model performances and chemical domain.

To calculate the distribution along the time scale of the pollutant in the environmental compartment the Fugacity Model EPISUITE (Level III Mackay) with default values was chosen.

In general, fugacity models predict the partitioning of an organic compound in an evaluative environment. A Level III model assumes steady-state but not equilibrium conditions. The Level III model in EPISUITE predicts partitioning between air, soil, sediment and water using a combination of default parameters and various input parameters that may be defined or estimated by other programs within EPISUITE.

The fugacity model in EPISUITE is a level III multimedia fate model using environmental parameters identical to those used in Mackay et al. (1992).

Like all level III models this is a steady-state non-equilibrium model. Steady-state conditions mean that the change in concentration of a chemical in each compartment with respect to time approaches zero. The model does not assume that a common equilibrium (fugacity) exists between the phases, so if a chemical is emitted into one compartment it can partition to the other compartments. Loss of chemical occurs through two processes: reaction and advection. Reaction is the biotic or abiotic degradation of the chemical that is calculated using the user specified or model calculated half-lives of the chemical in each of the 4 main compartments. Advection processes are considered for the air, water and

sediment compartments. Advection is the removal of chemicals from a compartment through losses other than degradation (reaction). The rate of advection in a given compartment is determined by a flow rate (m³/hour), calculated by dividing the volume of the compartment by an advection time.

2) ECOSAR version 0.99 h

The Ecological Structure Activity Relationships (ECOSAR) is a program used to estimate the toxicity of chemicals for the aquatic environment, created by the US EPA. It predicts the toxicity of industrial chemicals to aquatic organisms such as fish (it is not species-specific), invertebrates (Daphnid and Earthworms), and algae by using Structure Activity Relationships (SARs). It classifies the compounds into 42 classes identified by the presence of specific functional group. A single compound may be classified into one, two or more classes. The predictions are based on a class-specific linear correlation between the experimental toxicity and the partition coefficient n-octanol/water (K_{ow} , obtained by LogP Kowwin, CLogP). The software reports a warning for the prediction in case of logP exceeding defined limits and/or solubility issues.

3) ACD

ACD/ Freeware Suite is a suite of comprehensive tools for the prediction of basic physicochemical properties. It predicts pKa, logP, logD, aqueous solubility, and an array of molecular properties in seconds, within one interface, and simply from the chemical structure. The fragment-based models offer accuracy and cover a good breadth of chemical space, giving properties and behaviour of the compounds. It was used to compare values for LogP and Solubility and contains different training sets of compounds. It has the same fragment based predictive approach as EPISUITE, but some experimental values for pollutants are present in ACD and in EPISUITE.

4) DEMETRA

The DEMETRA project developed five, free models to determine the ecotoxicity of pesticides, as already detailed in the previous chapter.

It is a model developed under the DEMETRA project, founded by the European Commission to address the eco-toxicity evaluation of pesticides in a way suitable for the Directive 91/414 on pesticides. In the project five endpoints were considered:

- acute toxicity for Rainbow Trout (*Oncorhynchus mykiss*): LC50 96-hours exposure
- acute toxicity for Water Flea (*Daphnia Magna*): LC50 48-hours exposure
- acute oral toxicity for Bobwhite Quail (*Colinus virginianus*): LD50 14-days
- dietary toxicity for Bobwhite Quail (*Colinus virginianus*): LD50 8-days exposure and
- acute contact toxicity for Honey Bee (*Apis mellifera*): LD50 48-hours of exposure.

Due to the focus of the project for regulatory purposes, great attention was paid to avoiding false negatives.

There are also further rules (different for fish and daphnia) to be applied in order to reduce the error of the model.

5) CAESAR

In CAESAR new QSAR models specific for REACH have been developed for these endpoints:

- bioconcentration in fish,

- skin sensitisation,
- mutagenicity,
- carcinogenicity,
- developmental toxicity.

CAESAR's models aim to be transparent at the maximum level. To do this, all biological values, chemical structures, values of chemical descriptors and fragments, and algorithms developed within **CAESAR**, procedures are available on the **CAESAR** website.

In order to maximise the reproducibility, which is fundamental for models for regulatory purposes, to build the CAESAR models the following steps were set:

- checked if different tautomers gave different predicted results (this check is not typically done in QSAR modelling),
- used chemical descriptors based on two-dimensional structures (the use of three-dimensional structures typically requires manual optimisation of the conformation, which introduces variability),
- the modelling algorithms are publicly available (reference , defining and fixing all model parameters.

For the model validation CAESAR uses a wide series of statistical checks. External test sets are also used, to verify that the model performs correctly on new compounds. For the internal tests of QSAR models the Tropsha parameters were adopted.

6) SPARC

The SPARC program was created to address the gap in predictive chemical fate modeling. Regulatory mandates have created the need for efficient models for exposure and impact assessment of human and ecological systems to chemicals imposed directly or indirectly by human activities.

The SPARC system consists of an integrated array of modularized intra- and intermolecular interaction models that can be related (through the appropriate thermodynamic relationships) to a wide range of physical and chemical process parameters. The span in chemical parameter prediction (currently operational or under development) includes: (1) equilibrium constants for complex speciation (ionization and tautomerization) and interphase distribution (gas/liquid, liquid/liquid, solubilities) and (2) rate constants for reactivity (solvolysis and redox). Predictive capability extends to essentially any organic solute and derives strictly from molecular structure input. Solvents capability includes water and essentially any organic solvent or mixtures thereof. Reaction conditions (temperature, pressure, pH, and ionic strength) span ranges typical of environmental application.

7) TOXTREE version 1.51

TOXTREE is an application, developed by Ideaconsult Ltd., which is able to estimate toxic hazards by applying a decision tree approach. One of this is the Verhaar scheme for predicting the toxic mode of action. According to the original work of Verhaar et al., 1992, it assigns the analysed compounds into 4 categories on the basis of the molecular structure:

- Inert chemicals (substances which acting by mode of action of the narcosis or baseline toxicity)
- Less inert chemicals (substances acting by polar-narcosis mechanism)
- Reactive chemicals and
- Specifically acting chemicals.

The compounds that cannot be classified according to the previous rules are assigned to class 5.

3.3.3 Commercial software

Several commercial programs are able to calculate molecular descriptors or to predict numerous endpoints, such as carcinogenicity, mutagenicity, lethal dose for mammals, skin sensitization, aquatic toxicity, etc.

Among the tools available in my laboratory during my work I had the opportunity to use DRAGON to calculate descriptors and TOPKAT® to compare ecotoxicological endpoints.

Commercial software is often able to use a certain high level of modelling capability providing the user with a functional tool. The major problem encountered using such models is that they offer low transparency and only rarely are validation parameters and experimental training set values given. The procedure to obtain the final output is not always totally clear and the equations and procedures may vary among different versions of the same software.

1) DRAGON (version 5.5)

Dragon is an application for the calculation of molecular descriptors. These descriptors can be used to evaluate molecular structure-activity or structure-property relationships, as well as for similarity analysis and high throughput screening of molecule databases. DRAGON provides now 3224 molecular descriptors. The user can calculate not only the simplest atom type, functional group and fragment counts, but also several topological and geometrical descriptors. Some molecular properties are also calculated by the use of common models taken from the literature. Moreover, the Lipinski's alert together with drug-like indices is provided.

2) TOPKAT® version 6.1

It is a commercial software in which there are a series of individual models for assessing specific toxicological and ecotoxicological endpoints. For each compound a single model is chosen on the basis of chemical class. The output indicates if the compound analysed is in the dataset of TOPKAT, if it is in the OPS (Optimum Prediction Space) or it is in the OPS limits and if all the fragments are covered.

The software always gives a prediction of toxicity for a new molecule, but it in some cases may be accompanied by an error message. Scepticism about these models is related to the fact that the model may give good results for chemicals included in the TOPKAT ® dataset, but not for new chemicals that may contain a combination of critical chemical fragments.

3.4 Comparison of predictive models for ecotoxicity endpoints:

- **A) Invertebrate model (*Daphnia magna*)**
- **B) BCF**

A) Invertebrate model (*Daphnia magna*)

The performance of the predictive software TOPKAT®, ECOSAR and DEMETRA was examined in order to investigate the applicability and results for the invertebrate model (*Daphnia magna*).

Further references regarding this work are in Appendix 3, in the paper “Regulatory perspectives in the use and validation of QSAR. A case study: DEMETRA model for daphnia toxicity”, (Porcelli et al.2008).

An acute toxicity model towards water flea (*Daphnia magna*) has been used as a case study to outline a validation methodology compatible with regulatory purposes. Reliability, predictive power, uncertainty, and applicability evaluations have been verified with an external test set.

The evaluation has been done considering statistical parameters along with the nature of the errors. The DEMETRA model gave good statistical predictions, and the maximum error of the outliers was lower than those obtained with the other two models.

DEMETRA proved to limit the number of false negatives, when the use of its rules defined an acceptable uncertainty level.

A large set of compounds, not used in developing the model, was chosen, for further validation of the DEMETRA model predicting pesticide toxicity toward *Daphnia*. The predictions were satisfactory.

DEMETRA predictions were compared with those provided on the same endpoint by two popular models: ECOSAR and TOPKAT®. Neither of these is specific for pesticides, even though they both include a number of them. The predictions were not as good as with DEMETRA. This does not mean that these models are not appropriate for predicting other endpoints or compounds; for example,

TOPKAT® performs well for acute fish toxicity or bacterial mutagenicity (Moore et al. 2003; Cariello et al. 2002).

The present approach aims to compare the possibilities of a given model for assessing a new chemical. For more complex structures more complex models are necessary. Indeed, pesticides are complex structures, from both the chemical and the biological points of view, the latter being particularly important considering all their mechanisms of toxicity.

The DEMETRA model offers proof that complex heterogeneous chemical structures can be modelled together, and that outliers can be identified from a chemical point of view. The model can thus be used for several purposes, introducing safety factors specific for different chemicals. The DEMETRA model introduces criteria to assess prediction errors, both in extent and sign making it suitable for regulatory purposes.

A.1 Biological data and structure availability

The test set was organized by collecting data from the HAIR ecotoxicity database (HAIR 2006). This contains data for about 242 pesticides extracted from the German database of the *Federal Biological Research Centre for Agriculture and Forestry* (BBA).

An initial screening was done in order to avoid polymers, inorganic compounds, mixtures of molecules and mismatch between CAS number and name. Out of the remaining compounds there were collected data only on water flea (*Daphnia magna*) LC50 48h, which is the dose that kills 50% of the fleas after 48 hours exposure. Finally, we divided the subset into a new test set (135 compounds) and a set of compounds already used for DEMETRA modelling (74 chemicals). Acute toxicity values were converted to the negative of the logarithm of LC50. For each new compound the chemical structure was checked and downloaded from

the ChemIDplus web site (ChemIDplus Advanced 2006), then saved as an MDL mol file.

A first analysis compared data in the new database with those already used for the DEMETRA modelling. Only 16 of the 74 common compounds have identical figures in the two databases (probably the same experiment) while the other 58 compounds showed a correlation coefficient, R^2 , of 0.89 between the two series. Although this indicates a good correlation between the two databases, it is important to note that 15 of these figures differed by more than a factor of 4, and six by more than one order of magnitude. This is a major problem in building predictive QSAR models: the experimental values, i.e. the model input, are an intrinsic source of uncertainty. This can depend on the experimental procedure, the nature of the mechanism of toxicity, etc., and, as already mentioned, it is not possible to obtain a predictive model with less uncertainty.

A.2 Molecular descriptors

The three models require descriptors calculated on the basis of the two dimensional structure. The 16 chemical descriptors needed for the DEMETRA model (DEMETRA 2006) were calculated with the same version of the software used to build the original model: Dragon free version 3.0 (DRAGON 2003). To create the MDL mol file of all the molecules with explicit hydrogen the software OpenBabel v 1.0.0.1 was used (OpenBabel 2006). The same version of OpenBabel was used to generate the SMILES codes needed by the TOPKAT® and Ecosar models.

A.3 DEMETRA model

Once the descriptors matrix had been calculated, the tool available on-line at the DEMETRA web site (DEMETRA 2006) was used to predict the toxicity values.

This model for *Daphnia* is based on a training set of 220 compounds and the software was built through a hybrid model approach: the final model is composed of three individual models (one based on partial least squares and two on neural networks) joined in a mathematical function that leads them towards a single predictive value. A hybrid model integrates the results of the individual models in an intelligent optimized way, achieving a better prediction. This combines the strengths of each QSAR model and reduces false negatives.

Some restriction rules apply to identify outliers (Benfenati 2007). These rules, proposed by the DEMETRA consortium and generated by visual inspection, were taken into account and new considerations were added to define the two confidence limits better: compounds with a ratio of the observed and the predicted values in mg/L either more than 50 or between 50 and 15.

A.4 TOPKAT® model

In the case of *Daphnia magna* (DAPHNIA EC50 v3.1 model), four sub-models are available: alcohols, single benzene ring compounds, other aromatics and aliphatics; the original models are based on a training set of respectively 66, 101, 37 and 34 compounds. Only one model is automatically associated with a new compound, considering some distinctive fragments for each class.

With the prediction TOPKAT® performs and merges two kinds of pre-processing analysis: univariate analysis, the “Coverage Examination”, to establish whether all the structural fragments of the query structure are well represented in the training set, and multivariate analysis, called “Optimum Prediction Space -OPS- Examination”, to assess whether the query structure falls into the ensemble of good prediction, OPS, or into a border space called OPS limit. The two steps are summarized in a percentage confidence limit that reflects the information about the two analyses (Accelrys 2001). The software always gives a prediction of

toxicity for a new molecule but despite this feature in some cases an error message appears.

Batch processing is a feature of TOPKAT® v.6.1 but the results do not contain all information and warnings useful for evaluating the predictions so one-by-one processing was run parallel to the batch mode. The input file was a list of SMILES associated with the ID of the molecule from which TOPKAT® automatically calculates the descriptors the models need.

Five compounds of the BBA dataset were discarded; for three the error was because the SMILES input was not recognized by the software, while for the other two the descriptors could not be calculated because the compounds contained Sn.

The output text file indicates whether the compound is inside the OPS or the OPS limits, all fragments are covered and the compound belongs to the initial database used for training; finally the assessment is reported, with confidence limit (Gombar et al. 1995).

The manual specifies that a QSAR model is applicable only to query structures that fall inside or in the vicinity of the OPS (Accelerys 2001). Therefore, all the compounds inside the OPS and OPS limits (without considering the fragments coverage) were predicted and, for further details, prediction of the compounds inside the OPS and with all fragments covered was carried out.

Figure A.1

Experimental vs. predicted values of BBA compounds. Figure **A.1A**. DEMETRA prediction (135 compounds, $R^2=0.63$). Dashed lines represent an error of a factor of 50 between experimental and predicted values, dotted lines a factor of 15 in case of false negatives. Figure **A.1 B**. TOPKAT® prediction of compounds inside OPS/OPS limits without considering the fragment coverage (circles, 78 compounds, $R^2=0.02$) with predictions of compounds inside the OPS and with all fragments covered (crosses, 37 compounds, $R^2=0.30$). The six compounds in the training set are shown as black circles. Figure **A.1 C**. Ecosar prediction (127 compounds, $R^2=0.21$).

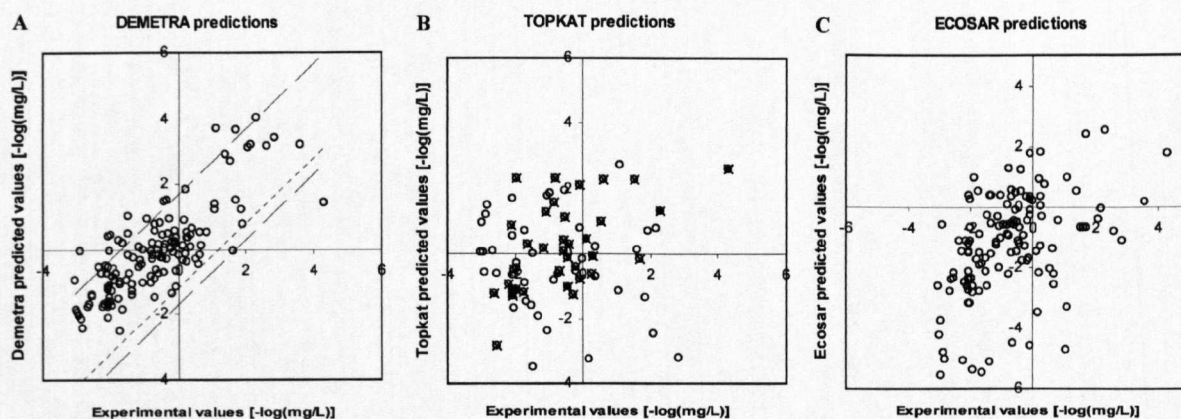
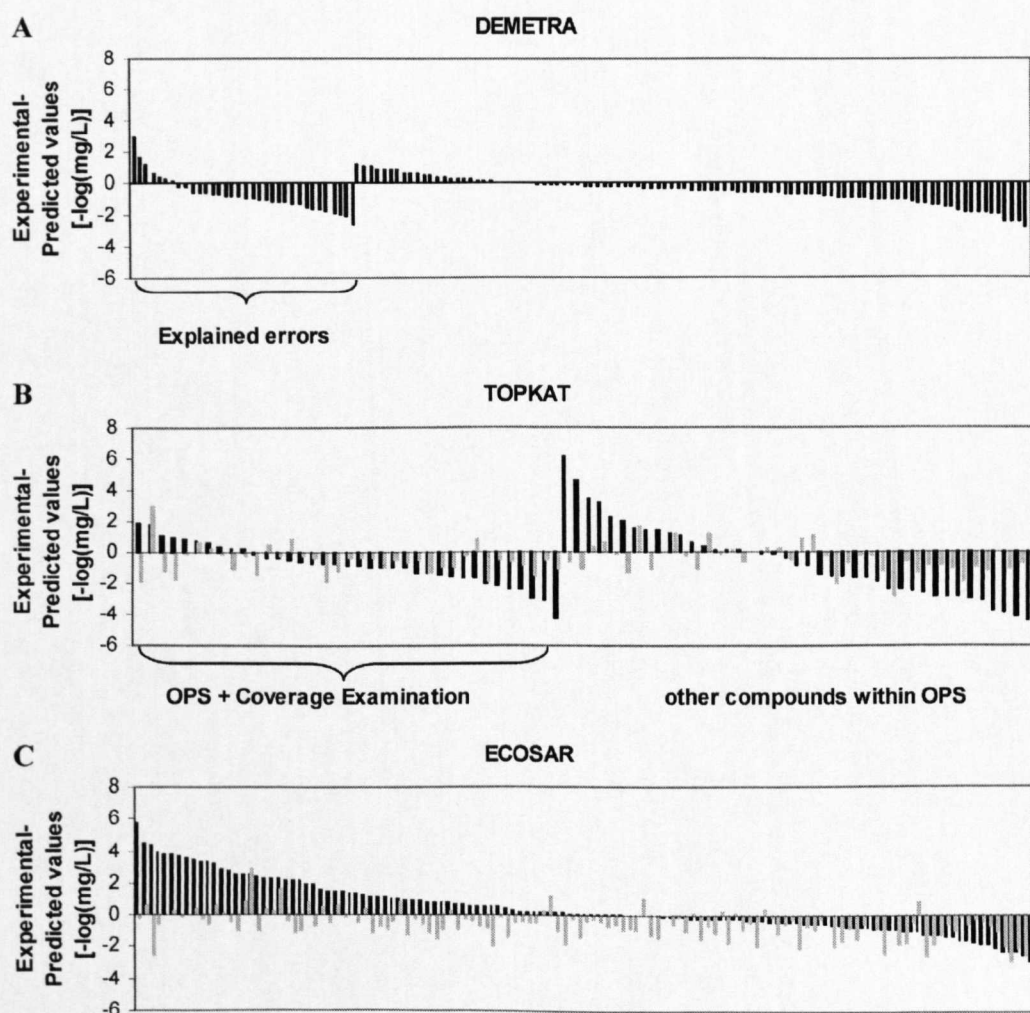


Fig. A.2

Errors for the predictions of the three models expressed as experimental minus predicted value

$[-\log(\text{mg/L})]$. Figure **A.2 A**. DEMETRA errors for each of the 135 compounds from the BBA database. The 34 explained errors are compounds outside the applicability domain using the DEMETRA rule-based approach. Range of not-explained errors 1.18/-2.87. Figure **A.2 B**. TOPKAT® errors (black) compared with DEMETRA errors (grey) of 78 compounds inside OPS/OPS limits without considering the fragment coverage, and 37 compounds inside the OPS with all fragments covered. Range of errors 6.08/-4.31 (1.85/-4.28 second case alone). Figure **A.2 C**. Ecosar errors (black) for 127 compounds compared with DEMETRA errors (grey). Range of errors 5.79/-2.86.



A.6 ECOSAR Model

The Ecosar program v0.99h is the computer version of the Ecosar procedure used by the US EPA Office of Pollution Prevention and Toxics (OPPT) for assessing new chemicals. The SMILES code is the input the program needs to classify a compound; individual QSAR models are associated with each class. The 62 chemical classes considered by ECOSAR are identified by the presence of distinctive functional groups. Whenever more than one class is found, human expert evaluation is required to associate the query structure with the right class, and consequently the correct QSAR model. Eight chemicals from the BBA test set were discarded because the software could not process the SMILES code or the chemicals were classified in a class for which no QSAR was available, as in the case of Imides.

Since ECOSAR is based on local models, the training set of each class contains fewer chemicals than the training set of DEMETRA.

The predictions of toxicity for new compounds rely on linear correlations of experimental toxicity values with their octanol/water partition coefficient, Kow. Kow for the test set was computed by KOWWIN, a program contained in the integrated tool EPI suite v3.12 (EPA 2006b). The range of Kow values valid for estimating the toxicity is given for each chemical class. If the log Kow is above than a certain cut-off the developers suggest that the lower solubility might affect the validity of the prediction and the QSAR models for longer exposure should be used to determine the LC50 (Clements 1996). This was taken into consideration in evaluating the models but gave no real improvement thus the results presented did not distinguish compounds with high log Kow.

A.7 Validation methods

The first classical QSAR studies were mainly interested in verifying whether some chemical features were related to a given biological effect. Today there is

more interest in the model predictive power than in simply unveiling such relationships. As a result, a battery of statistical tools has been introduced within the last few years to assess this predictive power. The classical QSAR models indicated mainly the fitting property of the model, given as R^2 . Nowadays it is accepted that the model predictive power has to be measured and reported, but there is a debate on the most suitable ways to measure this (Tropsha et al. 2003). A complicating factor is that different tools should be used depending on the model and the number of chemicals used to build it (Hawkins et al. 2003). Generally, leave-one-out validation is not considered suitable and a source of optimistic results, while good statistical results based on an external set of compounds, never used in the model building steps, are considered proof of the predictive power of the model (Golbraikh and Tropsha 2002).

DEMETRA models have been thoroughly validated by internal validation techniques, such as y-scrambling, leave-one-out, leave-more-out. Furthermore, DEMETRA adopted the criteria indicated by Tropsha et al. for the test set (43 compounds) prediction ($Q^2 > 0.5$, slope between 0.85 and 1.15, $(R^2 - R_{20})/R^2 < 0.1$) to evaluate the model robustness (Golbraikh and Tropsha 2002). The results on the training and test set, with all these criteria satisfied, were: $R^2_{\text{training}} = 0.74$, $R^2_{\text{test}} = 0.70$ (Benfenati 2007).

A.8 Performance

In the second phase a new set of 135 compounds produced within the HAIR project was obtained (HAIR 2006). The source of the toxicity values is reliable (the German BBA), but I did not repeated the quality check done before the modelling activities within DEMETRA, which involved checking the toxicity values with different databases, the purity and other parameters of the chemicals (Benfenati 2007). Figure 1A shows the results of the DEMETRA model for *Daphnia* toxicity. The R^2 on the HAIR test set is 0.63 (without applying any applicability domain rule). These results show that the DEMETRA model is a

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predictive model for pesticides. Overall, the number of compounds used for this exercise was about 80% of the training set – a particularly demanding percentage. Normally the validation uses a smaller proportion.

For a comparison, figure A.1B and A.1C show the results with TOPKAT® and ECOSAR. R² is 0.21 using ECOSAR and 0.30 using TOPKAT® (compounds in OPS and all fragments covered). The number of compounds is indicated in the legend.

The number of compounds which were outside the OPS and the OPS limits with TOPKAT® is 51; six were inside the training database and two presented further warnings indicating critical features (e.g. "Computed LogP Value Outside the Range Spanned by the Training Set"). ECOSAR detected 19 different chemical classes in the test set but almost half the compounds were classified as Neutral Organics. The classification was unequivocal for most of the chemicals though for 18 compounds the program assigned more than one class. When there are multiple residues it is up to users to decide the most appropriate model from their own experience, but this may result in lack of reproducibility. For these 18 chemicals with more than one possible model, the difference between the predictions of the models for the different classes was analyzed and no big difference was found in considering the more conservative one or the mean value among the two.

The main difficulty in the use of ECOSAR is the lack of specific QSARs for most classes of pesticides (Clements 1996). For instance no specific QSARs exist for carbamates, and, without any warning, they were classified as esters, amines or in the more general class Neutral Organic. Furthermore, the limited number of compounds in the training set of the ECOSAR models, and the dependence on a single factor (the only descriptor is logP) may explain the difficulty of modeling a large variety of compounds (Kaiser et al. 1999). Regressions based on logP are not predictive for pesticides, even considering only chemicals which should act

through narcosis, which is the theoretical assumption of the models based on logP (Hansen 2004; Sinclair and Boxall 2003).

Another important issue in case of a QSAR for regulatory purposes is transparency. DEMETRA is fully transparent: the toxicity data, chemical structures, descriptors and algorithms are publicly available, as is the source code (DEMETRA 2006). A detailed description of the modelling procedure has been published (Benfenati 2007). The transparency of the model and the data availability are important issues according to the OECD principles for validation of QSAR models for regulatory purposes (OECD 2004). Unfortunately, it is not always easy to obtain the data and the models, but this information is essential for a correct evaluation of the model. For instance, in the case of TOPKAT® six chemicals were present in the training set of the model. These are presented in figure A.3 B (filled circles); TOPKAT® appears to give better predictions for these chemicals than for the others in figure A.1B. This may be interpreted as overfitting of the model, meaning that for other compounds the model is not able to give similarly accurate results.

A.9 Modelling approaches

There are some differences between DEMETRA, ECOSAR and TOPKAT®. All three address heterogeneous chemical classes. DEMETRA actually more specifically addresses complex chemicals, such as pesticides and their metabolites, TOPKAT® includes some pesticides, while ECOSAR includes mainly simpler chemicals scattered in individual models.

The descriptors needed by the models can be generated from the 2D structure for all three cases, and this is a good feature because optimization of the 3D structure can involve a variable and time-consuming procedure. The difference is the nature and number of the descriptors: the chemical parameters of ECOSAR are fewer and TOPKAT® and DEMETRA introduce more sophisticated

parameters, which can be an advantage to describe the different components of the query structure better.

DEMETERA was designed to develop a battery of QSAR models combined within a hybrid model that uses the outputs (the predicted values) of the individual QSAR models as inputs. ECOSAR and TOPKAT® contain series of possible QSAR models, which work alternatively. Thus, these two do not integrate the results from the multiple modules they have. These multiple modules are structured following the same approach. ECOSAR and TOPKAT® theoretical reasoning is based on human expertise to classify compounds with similar fragments and then relate the chemical classes to descriptors which play a major role depending on the mechanism (see also Russom et al. 1996). Out of the multiple models, only one local model is activated, based on this theoretical assumption. The models therefore encode explicit knowledge which is used to identify chemical classes, giving the final result a degree of uncertainty.

DEMETERA does not introduce any human-based scheme but exploits modern knowledge discovery techniques. The assumption is that there is implicit knowledge in the data, and suitable information technology tools may extract this in an automatic and reproducible way. Various models have been developed using different approaches to produce as large as possible a basis for the final hybrid model.

Instead of focusing on a specific approach, the approach of DEMETERA preferred to screen large series of chemicals and mathematical tools. This strategy was used in several cases on hybrid systems for different applications and, from a theoretical point of view, there has been discussion as to whether it is more efficient than single methods (Neagu et al. 2005, Lemke et al. 2005). For wider discussion of the use and basis of hybrid systems in QSAR see Gini et al. 1998. DEMETERA hybrid models, as typical of hybrid systems, produced better results than any single individual model (Benfenati 2007). Furthermore, if different models produce different values, as with ECOSAR, the results may be conflicting,

and the user may be confused. A specifically optimised hybrid model can cope with this better, assigning different weights to different models.

A.10 Nature of errors

Besides the statistical parameters given above, it is important to assess the exact nature of the error given by the model (Benfenati 2007). Figure A.2 shows the positive and negative errors for DEMETRA, ECOSAR and TOPKAT®. The errors with DEMETRA are much smaller than with the other two. Large errors may pose a serious problem for the use of a model within a risk assessment procedure. Indeed, if such an error cannot be explained by rules that can be used for a new compound the regulator would prefer to apply a safety limit as large as the maximum error of a given model.

In DEMETRA some errors were larger for some chemical classes, such as carbamates, so they were allocated restrictive rules to warn the user in case of predictions for these chemicals.

A further facility in DEMETRA is that users can see the range of values predicted by the individual models at the basis of the hybrid model. Thus, they can choose, using not the final toxicity value of the hybrid model, but the most conservative value, which may be one of the values of the individual models.

As well as the size of the error, it is interesting to look at its sign. For toxicity prediction false negatives are much more critical than the opposite. Figures A.2 shows that ECOSAR and TOPKAT® give more false negatives. This is probably because the basic mechanism that is modelled best is narcosis, and deviations from this mechanism are not adequately codified. The DEMETRA model was developed designing the hybrid model to avoid false negatives. Different strategies have been developed and tested, using different mathematical tools, as explained elsewhere (Benfenati 2007). DEMETRA was focused closely on the regulatory use of the models, considering both the extent and the sign of the

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errors, besides the statistical validation tools which disregard this. Within DEMETRA, chemical-based rules were identified but other types can be obtained, for instance mechanistic ones (Benfenati 2007). Some outliers of the DEMETRA model can be explained by human experts on a biochemical basis, e.g. because they affect the electron transfer in the cell. But in this case the rule, even if sound because it is based on a known mechanism of toxic action, cannot be used to predict outliers, because at the moment it is not possible to predict whether a new chemical will act by the same mechanism.

B) BCF

The CAESAR model on BCF was compared with other freely available predictive tool as BCFBAF v3.00 (part of the EPISUITE toolbox) and LogP based equations. The CAESAR model has been designed to be suitable for REACH, considering the thresholds and legislative uses. Furthermore, innovative tools for a transparent check of the applicability domain have been developed and made publicly available through the web.

Further references regarding this work are in Appendix 3, in the paper "Assessment and validation of the CAESAR predictive model for Bioconcentration factor (BCF) in fish" (Lombardo et al.).

B.1 Biological data and structure availability

To build the CAESAR model a data set of 511 compounds together with measured logBCF values was taken from literature of Dimitrov et. al, 2005. According to the list of molecules' names and/or CAS numbers from the literature, the 2D chemical structures were checked using 5 different online databases: ChemFinder, ChemIDPlus, Safe Nite from Japan, Biodegradability Data and Estimate and PubChem Compound. Then, some compounds were omitted according to the following criteria: (1) compounds with lack of information

on the structure; (2) isomeric mixtures of compounds; (3) compounds presented as salts; (4) stereoisomeric mixtures; (5) metal complex compounds. Then, the final database was created with ISIS BASE 2.5 SP2 with 473 compounds. The data set covers a wide range of logBCF and logKow values (logBCF range from -1.00 to 4.85; logKow range from -4.3 to 12.66), with a molecular weight range from 68 to 943. Such a broad representation over the data space is important to ensure predictive capability of the QSAR models.

The 473 compounds have been sorted according to a hierarchical system of compound classes with respect to functional group. Within compound classes, the compounds were sorted according to halogen substitution, aromaticity, bond orders, ring content, and finally number of atoms. Particular attention has been given to proper ordering of compounds with mixed functional groups. From the sorted list, the test set has been separated by keeping the relations between these compound classes in both resulting sets as close as possible to the relations in the total set. The final training set included 378 compounds and the test set included 95 compounds.

After CAESAR modelling activities had started, two collections appeared, one from EURAS and one from Canada. So 172 new data were added as further validation after a careful check.

EURAS, as already reported in section 3.2.4, used only reliable BCF data for the fish indicated in the OECD 305 guidelines. Further checks were necessary to check the consistency of some exceptions (e.g DDT). For the Canadian database it was necessary to extract the more reliable BCF data (overall score of 1; endpoint 2: BCF-total water concentration) for the fish recommended by OECD 305 guidelines (*Danio rerio*, *Pimephales promelas*, *Cyprinus carpio*, *Oryzias latipes*, *Poecilia reticulata*, *Lepomis macrochirus*, *Oncorhynchus mykiss* and *Gasterosteus aculeatus*). For both datasets, all chemicals were further checked, verifying the chemical structure (searching and checking the SMILES code) using public databases online (ChemIDplus, PubChem Compound, Biodegradation and

Bioconcentration of the Existing Chemical Substances, EPA DSSTox Search Tool, IBM Chemical Search Alpha and InChI Converter). All the chemicals with too little information to find the structure, inorganic compounds, isomer mixtures, metal complexes and the data from experiments on mixtures of chemicals, were eliminated. The salts were neutralised. The chemical and experimental data at the CAESAR model are available on the CAESAR web site.

B.2 CAESAR model

The CAESAR model for BCF (Zhao et al. 2008) uses descriptors calculated using Dragon (Dragon v5.4) and MDL (MDL QSAR v2.2). The model combines results of two independent models, offering greater accuracy. The two models were developed using support vector machine (SVM). The program R and Matlab were used to build up the model.

B.3 LogP and its relationship with the BCF data

LogP is the logarithm of the partition coefficient between octanol and water. It is considered very important to assess the bioaccumulation potential of a substance. Most models use logP to predict BCF (alone or together with other descriptors). The guidelines of the European Chemicals Agency (ECHA) for REACH suggest using logP for screening (if $\log P < 4.5$, then the substance is non-bioaccumulative). Comparing the experimental values for logBCF and logP (see Figure B.1), experimental logP alone cannot separate compounds that are bioaccumulative or not. Table B.1 compares the results of the logP-based screening suggested by ECHA with experimental data. There are almost 2% of false negatives and the compounds with $\log P \geq 4.5$ are almost equally nB or B/vB (where nB means non-bioaccumulative, B bioaccumulative and vB very bioaccumulative). False negatives are compounds that are predicted as safe, without risky properties, but are in fact dangerous. Regulators want to avoid this

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situation. False positives are compounds that are predicted to be active, but are not.

The CAESAR model, like most of the QSAR models for BCF, uses mainly log P as a fundamental descriptor. So it is quite similar to models like BCFBAF v3.00 and many others. For the comparison I used a series of log P values calculated with four programs at pH 7. Table B2 reports the correlations between these calculated log P values and the experimental BCF, for the chemicals used in the CAESAR model. When the model was developed, it also used logD as an additional descriptor, calculating the partition coefficient in a series of acid and basic pH, but the results were no better.

As shown in Table B2, the correlation between log P and BCF is not enough to support the use of this single parameter with a simple model. This is the same message as in Figure 1B, where experimental values were used.

Table B1 Confusion matrix for logBCF classification using logP experimental values.

		logP	
		< 4.5	≥ 4.5
logBCF	nB1	70.48%	14.32%
	B2	1.10%	3.08%
	vB3	0.44%	10.57%

1 Non Bioaccumulative

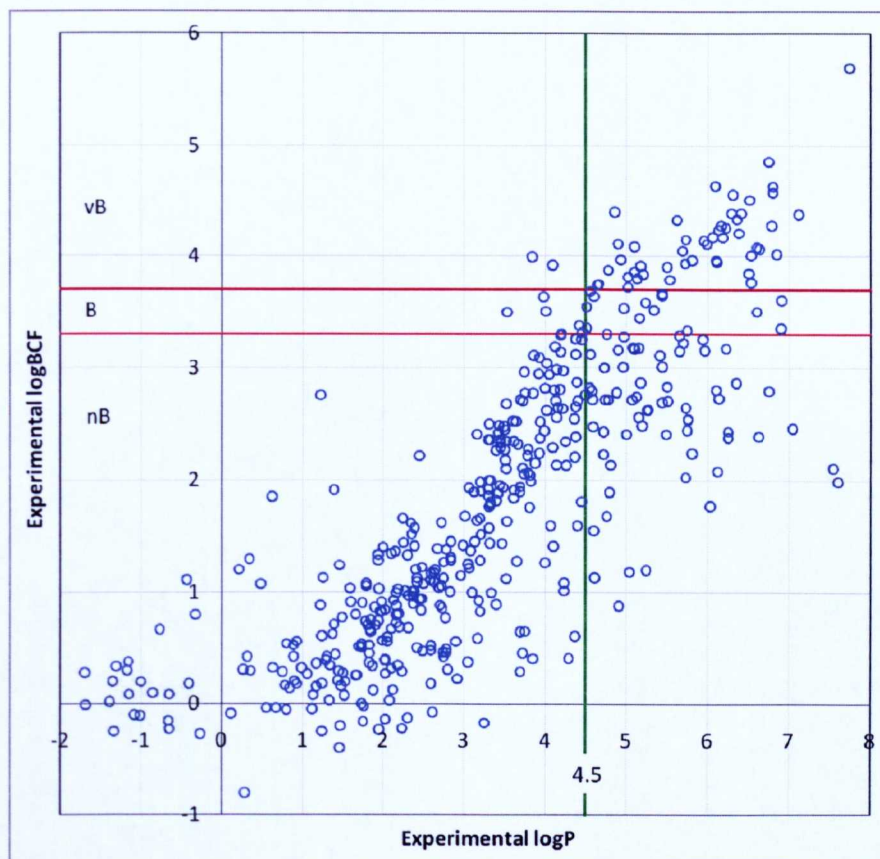
2 Bioaccumulative

3 Very Bioaccumulative

Table B2 Regression coefficient between logP calculated with different programs and BCF.

Descriptor	Chemical meaning	Source	Model	R	R 2	F value
logPACD	logP value calculated by ACD software	ACD software	logBCF=0.305* logPACD +0.767	0.605	0.336	217.442
logPKowin	logP value calculated by Kowwin software	Kowwin software	logBCF=0.357* logPKowin +0.605	0.657	0.432	266.931
logPMDL	logP value from MDL descriptors	MDL descriptors	logBCF=0.481* logPKowin +0.290	0.737	0.543	448.043
MLOGP	Moriguchi octanol-water partition coeff. (logP)	Dragon software	logBCF=0.555* MLOGP +0.117	0.746	0.556	471.748

Figure B1. Comparison of experimental values of logP and logBCF. The two thresholds for BCF indicated in the REACH legislation are shown. The screening threshold proposed by ECHA for logP 4.5 is also reported.



Different factors are indeed involved in the BCF process and further descriptors are necessary to simulate this better. Thus, seven other descriptors were identified using powerful information technology tools to screen a large number of potentially useful descriptors. Here the term descriptor is used in its broadest sense, including molecular descriptors and fragments. Existing software, like the programs used and described in the experimental section, can calculate a large number of descriptors, considering the molecule as whole, or counting smaller molecular parts, like atoms or molecular groups. In this way, the model can be improved by extracting knowledge related to molecular descriptors, which boost its performance, taking account of other molecular features related to the property of interest. The CAESAR model includes two independent models, which run in parallel, and the results are combined in an integrated model .

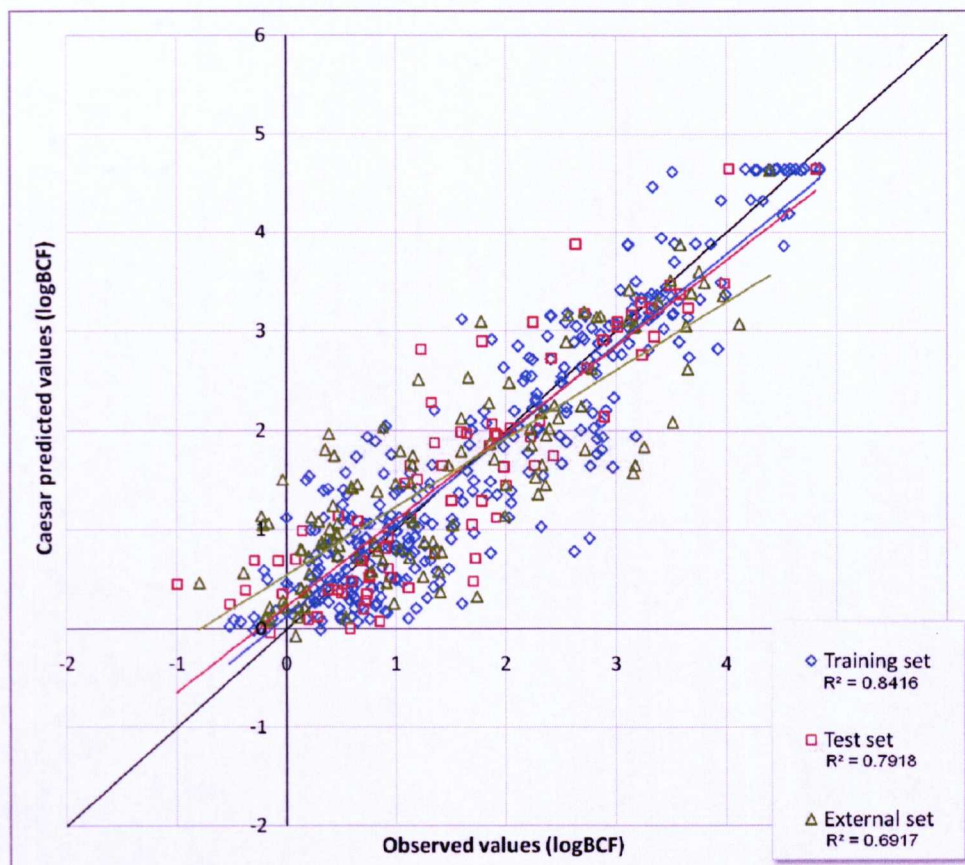
For this comparison only experimental logP values—obtained from the Arnot database and the internal database of KOWWIN v1.67 (included into EPISuite v4.0) were used. The experimental BCF values used for the comparison were obtained from two sources (EURAS, Zhao et al. 2008). When two different logP or BCF values were reported, the average was used. In total, 454 compounds (from the 635 available) had an experimental logP value and were tested.

B3 Validation of the CAESAR model

A major criticism of QSAR models is that they reflect the current list of chemicals used to build up the model, but they cannot always predict the values for new compounds. For this reason, great care is needed in validating the QSAR model, using good statistical methods. There is still a debate in the QSAR community on the best ways to verify whether the model is predictive or not. Some authors prefer external validation, which is done with a set of compounds never used during the development of the model. This approach is questioned by others, who note that in some cases the number of compounds is too limited to use this approach without renouncing a significant proportion in order to represent the real situation; furthermore, external validation is related to the specific list of compounds, which can represent a bias. Thus, other methods are suggested, preferring internal validation.

Figure B2 shows the results of the BCF models on the training set, on the first validation set and on the new, second validation set.

Figure B2. CAESAR model performance. Comparison of the experimental logBCF values and the predicted ones using the CAESAR model (chemicals within the applicability domain), for the training, validation and external sets.



The standard deviation error in prediction (SDEP) of the CAESAR BCF model is about 0.5.

The SDEP was calculated according to:

Eq (28)

$$SDEP = \sqrt{\frac{\sum(o_i - p_i)^2}{n}}$$

where o_i are the observed values, p_i the predicted values and n the number of values.

Performances comparison of the models

The 635 compounds that form the complete dataset used for this work were split into training (370 compounds), first validation (93) and external sets (used as the second validation set of 172 compounds) of the CAESAR model. Because some of them are not in the applicability domain of the model, the three sets were reduced to 327, 81 and 119 compounds respectively. The percentage of the total compounds predicted is given without considering those that are outside the applicability domain. To get more conservative results, all the compounds near the two thresholds for B and vB compounds were raised 0.5 log units. To do this compounds between 2.8 and 3.3 were predicted as 3.31 and compounds between 3.31 and 3.7 as 3.71. Table B4 shows this modification.

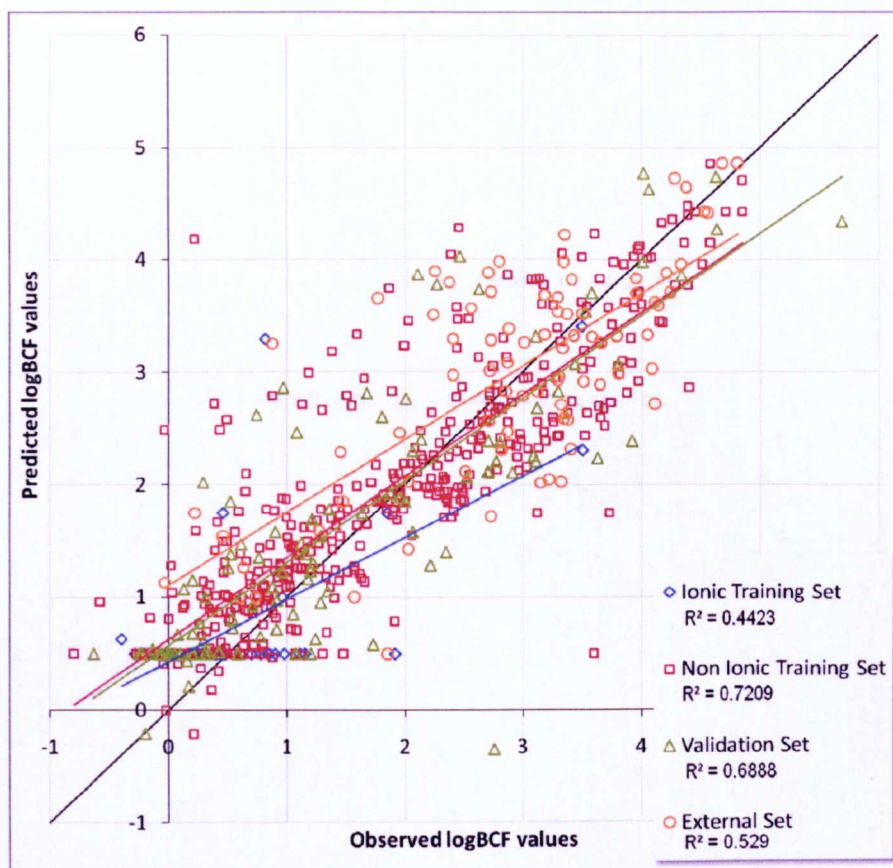
Similarly, we split the 635 compounds into a training set of 103 compounds (as indicated by BCFBAF v3.00), and an external set of 82 compounds, (never used by BCFBAF v3.00). The results were analysed, yielding the confusion matrix reported in Table B.5 and B.6. In this case only one compound was outside the applicability domain of the model (defined from the molecular weight and logP), but it is well predicted, so it was not eliminated.

This is in agreement with the variability of the experimental data, and shows that on average the expected errors of the *in silico* and experimental methods are similar. The following results consider only the compounds within the applicability domain of the CAESAR model, 527 in total. The overall R² (the square correlation coefficient between predicted and experimental values) is 0.81 for CAESAR model, and the R² for the second validation set is 0.69. The SDEP is 0.57 for all the compounds, and 0.70 for the second validation set. To evaluate the performance of the model better we also considered the Q² (calculated according to Schüürmann et al, 2008) of the entire dataset and of the external one, and the results were practically identical to R², showing that the CAESAR model is predictive even if there is a reduction of the statistical characterisation.

The overall R^2 is 0.75 for BAFBCF v3.00 model , and the R^2 for the second validation set is 0.79. The SDEP is 0.68 for all the compounds, and 0.81 for the second validation set.

Figure B3 shows the performance of the BAFBCF v3.00 model reporting the results for the compounds used by the developers in their validation and training sets.

Figure B3. BCFBAF v3.00 performance. Comparison of the experimental logBCF values and the predicted ones using the BCFBAF v3.00 model for the ionic training, non-ionic training, validation and external sets.



BCFBAF v3.00 split chemicals in ionic and non-ionic. The developers did not use the compounds in the external set during the model development. This set consists of 82 compounds and many of the compounds were already present in the BAFBCF v3.00 training set. Compared to the 450 compounds in the BAFBCF v3.00 training set, the number of compounds (82) in this external validation set amount to 18%. The performance was also checked, using the three splits (training, validation done by the developers, second validation with new compounds), and comparing the results for these splits according to CAESAR and BCFBAF v3.00. This meant the comparison was not biased by one splitting procedure, because all possibilities were assessed. Table B2 shows the results, indicating the R^2 and the SDEP. We excluded compounds that CAESAR labels as unreliable.

Table B2. R^2 and SDEP for CAESAR and BCFBAF v3.00 models. R^2 , SDEP and number of compounds are reported for both models for the following sets of compounds: CAESAR (training, test and external), BCFBAF v3.00 (training, validation and external), compounds shared between CAESAR validation and BCFBAF v3.00 validation, compounds shared between CAESAR validation and BCFBAF v3.00 external and total compounds. Only the compounds in the applicability domain of CAESAR were analysed.

Set	CAESAR training		CAESAR test		CAESAR validation	
Model	CAESAR	BCFBAF v3.00	CAESAR	BCFBAF v3.00	CAESAR	BCFBAF v3.00
No. values	327	327	81	81	119	119
R^2	0.85	0.80	0.83	0.77	0.69	0.61
SDEP	0.53	0.62	0.51	0.61	0.70	0.85
Set	BCFBAF training		BCFBAF validation		BCFBAF external	
Model	CAESAR	BCFBAF v3.00	CAESAR	BCFBAF v3.00	CAESAR	BCFBAF v3.00
No. values	383	383	80	80	64	64
R^2	0.79	0.76	0.78	0.67	0.79	0.79
SDEP	0.57	0.64	0.61	0.75	0.49	0.81
Set	CAESAR validation \cap BCFBAF v3.00 validation		CAESAR validation \cap BCFBAF v3.00 external		total	
Model	CAESAR	BCFBAF v3.00	CAESAR	BCFBAF v3.00	CAESAR	BCFBAF v3.00
No. values	22	22	7	7	527	527
R^2	0.74	0.68	0.61	0.19	0.81	0.75
SDEP	0.64	0.69	0.72	1.25	0.57	0.68

B4 Classification approaches for BCF

Using a quantitative model like CAESAR as a basis for classification of BCF has the main advantage that its use remains flexible, not linked to a specific threshold, such as those indicated in specific legislations. For instance, a substance is considered bioaccumulative for REACH if the BCF value is greater than 2000, but for CLP the threshold is 500. Therefore, it can still be used if these limits are modified or updated over the years. In this section, the CAESAR model according to the REACH classification was analysed. Depending on the tonnage of the chemical to be put on the market, REACH specifies different ways to report the BCF characterisation. As already explained, for lower tonnage the information is only categorical, to define the chemical as bioaccumulative or not; however, at higher tonnage (> 100 tonnes/y) BCF has to be given as a continuous value to be used for risk evaluation.

Table B3 shows the results of the model, used for classification in three classes with the B and vB limits indicated in REACH: 3.3 in log units for B and 3.7 for vB. To take account of the uncertainty related to experimental and predicted values, an offset of 0.5 log units was applied to the compounds whose predicted BCF values fell near the B and vB thresholds. In other words, a conservative criterion was applied, reflecting the fact that the data are affected by a given uncertainty.

Table B3. Classification with the CAESAR model. Three sets are reported: training, first validation and second validation set. The percentage of the total of compounds predicted is given without considering those outside the applicability domain. In brackets, the number of compounds for each class. The total number of compounds is also reported.

<i>Training set</i>		<i>Observed logBCF</i>			<i>First validation set</i>		<i>Observed logBCF</i>			<i>Second validation set</i>		<i>Observed logBCF</i>		
327 comp.		nB	B	vB	81 comp.		nB	B	vB	119 comp.		nB	B	vB
Predicted logBCF	nB	82.46 (270)	3.38 (11)	0.31 (1)	Predicted logBCF	nB	90.00 (72)	3.75 (3)	0.00 (0)	Predicted logBCF	nB	88.24 (105)	4.20 (5)	0.84 (1)
	B	1.54 (5)	2.15 (7)	0.92 (3)		B	0.00 (0)	1.25 (2)	1.25 (1)		B	0.84 (1)	1.68 (2)	2.52 (3)
	vB	0.62 (2)	1.23 (4)	7.38 (24)		vB	1.25 (1)	0.00 (0)	2.50 (2)		vB	0.00 (0)	0.84 (1)	0.84 (1)

Table B4. Classification with the CAESAR model. Three sets are reported: training , first validation and second validation set. The percentage of the total of compounds predicted is given without considering those that are outside the applicability domain. In brackets, the number of compounds for each class. The total number of compounds is also reported. To take account of the endpoint variability, the predicted values are modified adding an offset of 0.5 log units for the compounds near the B and vB thresholds.

Training set		Observed logBCF			First validation set		Observed logBCF			Second validation set		Observed logBCF		
327 comp		nB	B	vB	81 comp.		nB	B	vB	119 comp.		nB	B	vB
Predicted logBCF	nB	73.70 (241)	0.31 (1)	0.00 (0)	Predicted logBCF	nB	77.78 (63)	0.00 (0)	0.00 (0)	Predicted logBCF	nB	81.51 (97)	1.68 (2)	0.00 (0)
	B	0.00 (0)	0.00 (0)	0.00 (0)		B	0.00 (0)	0.00 (0)	0.00 (0)		B	7.56 (9)	1.68 (2)	0.84 (1)
	vB	11.01 (36)	6.42 (21)	8.56 (28)		vB	12.35 (10)	6.17 (5)	3.70 (3)		vB	0.84 (1)	2.52 (3)	3.36 (4)

In Table B3, it is underline that when used as a classifier the CAESAR model has clear advantages over the single criterion of the logP at 4.5 (see above) because: 1) it can predict three classes; 2) the accuracy of the prediction is much higher (always above 90% even on the second validation set, while accuracy for logP as from Table B1 is about 84%). Table B4 shows the confusion matrix using the CAESAR model as a classifier, with the 0.5 offset explained above. The percentage of false negatives decreases, but false positives increase. This solution is more conservative, as explained.

The performance in classification of the CAESAR model (without and with the 0.5 correction) was compared with that of BCFBAF v3.00 (see Tables B5 and B6). Figure B4 shows the comparison of the accuracy of the models.

Figure B4. CAESAR and BCFBAF v3.00 accuracy. Comparison of the accuracy, using CAESAR and BCFBAF v3.00, for their three respective sets (training, validation and external). * Modified: using an offset of 0.5 for values close to the thresholds (see text).

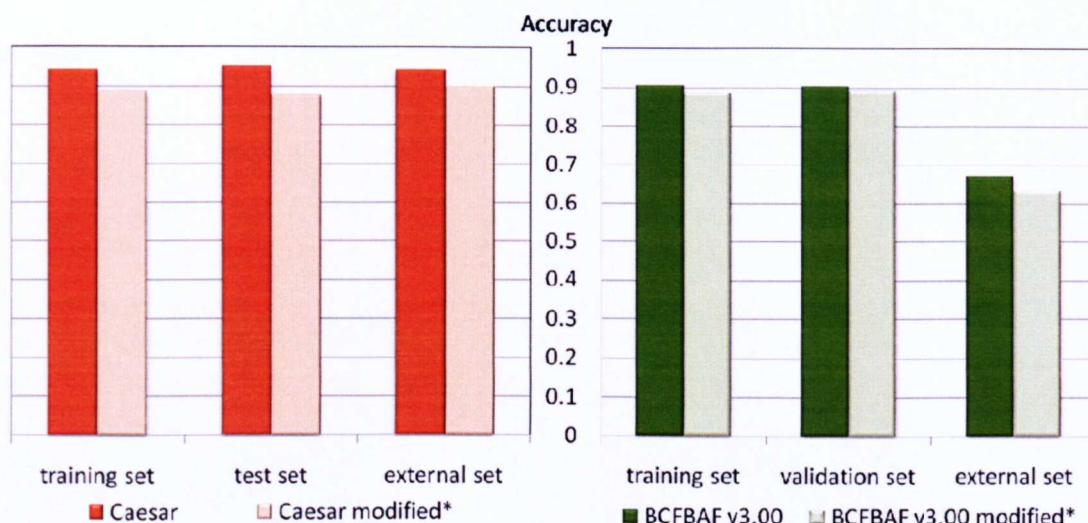


Table B5. Classification with the BCFBAF v3.00 model. Three sets are reported for the compounds of the dataset: training, validation and external. The percentage of the total of compounds predicted is given without considering those outside the applicability domain. In brackets, the number of compounds for each class is reported. The total number of compounds is also reported.

Training set		Observed logBCF			Validation set		Observed logBCF			External set		Observed logBCF		
450 comp.		nB	B	vB	103 comp.		nB	B	vB	82 comp.		nB	B	vB
Predicted logBCF	nB	82.00 (369)	3.33 (15)	2.00 (9)	Predicted logBCF	nB	81.55 (84)	2.91 (3)	1.94 (2)	Predicted logBCF	nB	39.02 (32)	12.20 (10)	3.66 (3)
	B	2.00 (9)	0.67 (3)	2.00 (9)		B	0.97 (1)	0.97 (1)	0.00 (0)		B	10.98 (9)	4.88 (4)	4.88 (4)
	vB	2.22 (10)	0.89 (4)	4.89 (22)		vB	3.88 (4)	0.97 (1)	6.80 (7)		vB	6.10 (5)	3.66 (3)	14.63 (12)

Table B6. Classification with the BCFBAF v3.00 model. Three sets are reported for the compounds of the dataset: training, validation and external. The percentage of the total of compounds predicted is given without considering those that are outside the applicability domain. In brackets, the number of compounds for each class. The total number of compounds is also reported. To take account of the endpoint variability, the predicted values are modified adding an offset of 0.5 log units for the compounds near the B and vB thresholds.

Training set		Observed logBCF			Validation set		Observed logBCF			External set		Observed logBCF		
450 comp.		nB	B	vB	103 comp.		nB	B	vB	82 comp.		nB	B	vB
Predicted logBCF	nB	77.56 (349)	2.22 (10)	0.44 (2)	Predicted logBCF	nB	78.64 (81)	1.94 (2)	0.97 (1)	Predicted logBCF	nB	26.83 (22)	6.10 (5)	1.22 (1)
	B	0.00 (0)	0.00 (0)	0.00 (0)		B	0.00 (0)	0.00 (0)	0.00 (0)		B	0.00 (0)	0.00 (0)	0.00 (0)
	vB	8.67 (39)	2.67 (12)	8.44 (38)		vB	7.77 (8)	2.91 (3)	7.77 (8)		vB	29.27 (24)	14.63 (12)	21.95 (8)

B5 Discussion

The BCF model developed within the CAESAR project proved to be predictive on the basis of a second validation set of 119 compounds, showing the robustness, reliability and predictivity of the model. Indeed, the second validation set is larger than the first, and its population is expected to be more heterogeneous. Conversely, the new set of validation compounds included all compounds for which new data were found from the sources mentioned, and is thus probably more heterogeneous than the first validation set, which had the same data source.

Using the second validation set, the SDEP is still comparable with the experimental variability, which range from 0.75 to 0.42 for the sets of substances used (see above). The limited increase of SDEP is partially due to this experimental variability, and partially to the model.

In conclusion CAESAR model features can be summarised with the following points:

- Reliability and transparency

All data used to build up the model, all structures, and the algorithm are given; the algorithm has been detailed in the scientific literature, including a description of the code (Zhao et al., 2008) and the structures and data are publicly available through the web. The model starts from experimental data obtained following an official protocol documented and suitable for REACH. All chemical structures have been checked within CAESAR by at least two partner laboratories, and a series of compounds have been eliminated, for errors or lack of sufficient detail in the structure or experimental protocol. This shows the very high quality evaluation of the input data. Furthermore, the output of the model has been designed for use with REACH, keeping in mind the thresholds given by this

legislation. The model has been optimised to reduce the number of errors, particularly false negatives. This proves that the model is suitable for the output specifications, for classification and labelling and risk assessment, as required by REACH.

- Applicability domain:

it means assessing whether the model, even if good from a general point of view, is suitable to be applied to the specific chemical of interest. For BCF we developed a series of independent tools to assess this:

- Chemical descriptor space. For instance, we excluded carbon disulphide because CAESAR reported the descriptors were out of the range.
- Rules, codified into structures that lead to greater uncertainty; they are identified by CAESAR using SMARTS (SMiles ARbitrary Target Specification). For instance, CAESAR identified a potential problem with a compound containing silicon.
- Visualisation of similar substances. A tool was developed for this, showing the six most similar compounds in the training set.
- Measurement of the similarity. These six similar compounds are also related with a numerical score indicating the similarity with each compound of interest. The approach and algorithm are described in the experimental section. For instance, carbon disulphide had a poor similarity value, lower than 0.5.
- The predicted value for each of these six similar compounds is reported, compared with their experimental value, to give a direct appreciation of the potential errors.

Thus, there were developed new tools for applicability domains, offering users information to assess whether a prediction is reliable for a certain compound. This battery of approaches for the applicability domain is innovative and complex. It uses not only *a priori* tools, based on chemometric measurements, as other methods do but we have added rules *a posteriori*, based on our results. Thus, these give a further evaluation, not only theoretical on the basis of chemical

descriptors and fragments, but also on the basis of the output values and the observed errors.

These tools to identify pitfalls may help to explain why CAESAR performs better than BCFBAF v3.00. This latter identified only one substance potentially outside the applicability domain. Figure B3 shows the performance of CAESAR using all possible splits of chemicals. The R^2 is always slightly higher than that of BCFBAF v3.00, and the SDEP, which shows the error, is always slightly lower. In one case the two models perform at the same level.

The user should always check and carefully evaluate the information given by CAESAR on the applicability domain. If there is a warning (for the range of descriptors or for the presence of critical fragments), or if the similarity of the chemical is not satisfactory, or if there are errors in the prediction of similar compounds, these factors should all lead to the conclusion that the model is not reliable for the chemical under evaluation.

If these factors are excluded, we can expect the error to be of the same order of magnitude as the experimental error. Further concern may arise when the predicted value is close to the threshold.

CHAPTER 4: Use of the Index in various real case studies as a tool to classify the status of a territory

4.1 Introduction:

ERICA's main features are to provide "pictures" of the global healthiness state of sites. In particular, it is possible to use ERICA as an instrument for prioritizing the hazards of specific polluted areas. Furthermore, ERICA provides a general risk assessment procedure useful to compare normative instruments based only on legislation parameters and chemical compounds recommended by legislation.

In order to validate the ERICA approach and evaluate its robustness, it was applied to two case studies. The first one was a dedicated scenario based on Italian legal limits and quality objectives for environmental matrices (air, water and soil). Then a pilot case was set up on the surrounding area of a landfill site located near the Italian westcoast.

4.2 Italian legal limits and quality objectives:

In the tables 10, 11, 12, 13 (**Appendix 2**) the main Italian legal limits and quality objectives set for the future for some compounds not yet under legislation are reported.

In order to validate ERICA these data were chosen because they represent the upper limit of risk management. For some compounds (e.g. TCDD in soil) the Italian limits are very strict due to the Seveso accident and political agreement (USEPA, 2009). For other compounds (e.g. benzene in air) there is still no fixed legal limit but a quality objective.

There are few publications related to the relationship between risk assessment values and law limit values because law limits are the results of political, social, economical and country specific strategies that for some contaminants leads to different results compared to the simple risk assessment procedure.

Other values, like for example Soil Screening Values (SSV), are often then used as quality standards adopted in many countries to regulate the management of contaminated land. They are usually in the form of concentration thresholds (mg/kg soil-dry weight) of contaminants in soil above which certain actions are recommended or enforced.

The implications of exceeding the soil SVs vary according to national regulatory frameworks. They range from the need for further investigations to the need for remedial actions. Along with their various roles in national regulatory frameworks, soil SVs have been given various names in different countries and they are called trigger, reference, target, intervention, clean up , cut-off, and many others.

Thus, the strength of enforcement varies. In some countries the reference to generic screening values is obligatory and the derivation of alternative values based on site-specific risk assessment is possible only under certain conditions. In other countries screening values are provided to risk assessors with generic guidance on the significance of contaminant concentrations in soil in a first tier of investigation, followed by a site-specific risk assessment in a higher tier (Carlson et al., 2007).

4.3 Landfill managements in Italy, a brief introduction

Landfill management is described as the operations carried out in the landfill necessary to provide the waste disposal service, and the consumptions and emissions generated by these operations. Landfill management also includes construction (excavation of the site and its preparation) and post-closure operations (site remediation and environmental requalification of the landfill).

To date, the Italian approach towards waste management has mainly been slow and geared towards short-term solutions. Only in 1997 the Italian state with the legislative decree n.22/97 (Decreto Ronchi) transposed the Waste Directive (1991) and the EU Packaging and Packaging Waste Directive (1994) into national law, grasping the opportunity of introducing major changes into its system for waste disposal and management. One of the salient features of the Decreto Ronchi is that, from the year 2000, landfilling has been only acceptable as a disposal option for inert waste and treated residues. This imposed stricter controls to waste sent to landfills, in particular biodegradable waste, and caused changes in waste management, including waste dry-wet separation and biological treatment of the organic material before its dumping.

In the considered landfill the waste pre-treatments occur after waste weighing and before their being sent to the 'dumping front'. Here, waste or its treated residues are compacted and covered with an inert, recovered material layer or with topsoil, about 20 cm thick, in order to limit the waste surface exposure to the atmosphere, to minimise possible odour emissions and to set up a suitable foundation for vehicle transit. (Binaghi et al., 2005)

4.4 Case Study 1: use of the Index in real case studies as a tool to classify the health of a territory

For this pilot case a detailed evaluation of the risk was available. The evaluation was done by our laboratory following Italian legislation guidance (VIS: valutazione impatto sanitario/ evaluation of healthiness impact). The possible hazards posed by contaminants were assessed so the results of the index and VIS outputs are compared.

4.5 Description of pilot case 1

This investigated site is located in the municipal area of Savona (Italy), in the Boscaccio landfill, situated in the Valley of Segno, about 5 km north-west of the Municipality of Vado Ligure. Waste transfer to Boscaccio started in 1992 at an elevation of 374 m above sea level. In the 1992–2002 period, 645,826 tons of waste was disposed of in this landfill.

In those years, the company managed an increasing amount of waste, starting from a minimal quantity of 30,000 t/year up to 100,000 t/year.

Leachate is collected in two storage tanks with capacities of 100 m³ and 400 m³ respectively. Each tank is provided with a lift station for leachate recycling on the landfill body. Using perforated pipes, waste in the landfill body is sub-irrigated. This operation has a twofold function: it leads to better waste compacting and it supports the waste fermentation process, speeding up digestion of the present organic fraction. Excess leachate is discharged in a sewer system.

The biogas produced by the biological processes that occur in the landfill is collected by 32 biogas extraction wells, linked in groups to biogas sub-stations, acting as regulators of a collecting process. The collected biogas is sent to 4 engines (3 engines with 330 kW power and one with 240 kW power) for energy recovery (electric) from biogas. If the cogeneration plant stops, the collected biogas is automatically sent to a flare. This flare operates as a safety valve of the whole plant. In 2002, it operated for about 67 hours. In 2002, 98.8% of collected

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biogas was sent to the engines, for a resulting production of 6,235,107 kWh, corresponding to 1.55 kWh/Nm³. The estimated biogas collection efficiency is 70%.

4.6 Monitoring organization and analysis procedure

The samples to study the risk posed by the landfill in the surrounding area were collected in the period 22 January 2009 to 13 March 2009 in the municipality of Vado Ligure and Bergeggi. This gave the possibility to study all the possible affected areas by the landfill emissions. Nine points were chosen at which to collect the samples as described in Fig 4.1:

Fig 4.1: area under study and points where the samples were collected



- P1: Landfill, offices
- P2: Landfill, nearby leacheate disposal
- P3: Cave entrance
- P4: Na Vaè;
- P5: Sport facilities centre – horses
- P6: San Genesio;
- P7: Beach, c/o dogane;
- P8: Offices EcoSavona;
- P9: Bergeggi, Botanical garden.

The analyzed pollutants are: dioxins, PCB, PAH, metals in the depositions, in environmental air (with sample of medium and high volume) and in soil; benzene, toluene, ethylbenzene, xylene, ammonia, NO₂, SO₂ in environmental air (using Passive Sampling-Radiello); O₃ using data from the Italian Environmental Agency detected in Vado Ligure; particulate matter (PM₁₀ and breathable fraction, PM₄); VOC in biogas, in the ground water and in particulate matter.

4.7 Methods to calculate ERICA:

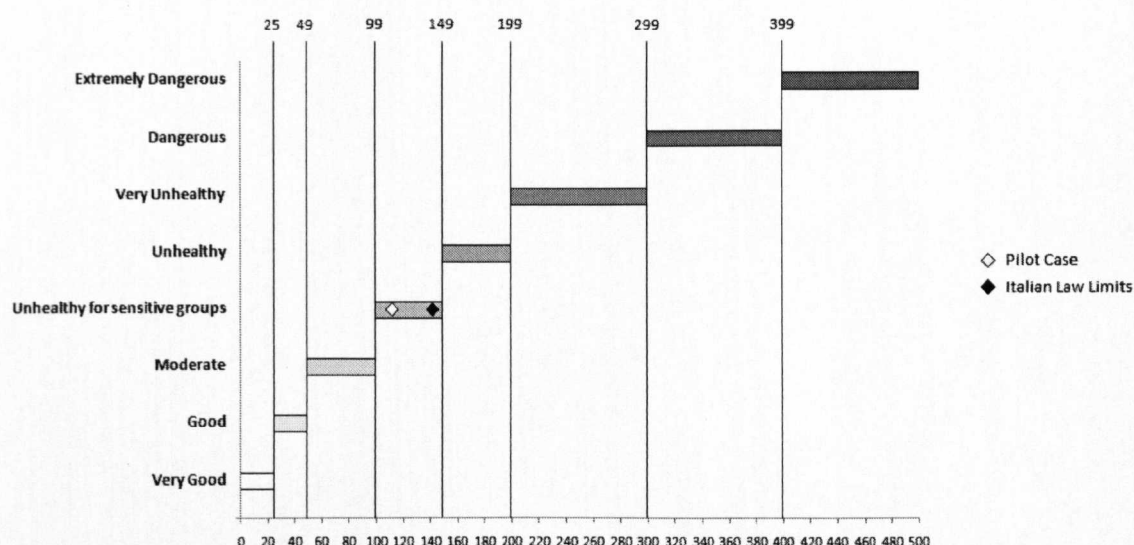
In case of missing input data (no set limits for some pollutant or no available samples) we set SRI equal to the risk threshold (= 1), as previously described.

Sub-indexes and final ERICA values for both case studies are reported in Table 4.1 and Fig 4.2.

Table 4.1 Sub-indexes and final ERICA values for the pilot case and for the law limit case.

Case Study	Pollutants	Soil				Water				Air				EQI	HQI	HCR	SRI	SRI limit	PI	EF	EFI	ERI	<ERI>	ERIE	ERICA	
		SEQ	SHQ	SHC	D	SEQ	SHQ	SHC	D	SEQ	SHQ	SHC	D													
L a w	Arsenic	16	32	32	4,17	4	4	32	4,17	0,5	1	16	4,17	83,35	154,17	333,33	163,55	100,00	1,64	19,00	1,65	270,38				
	Cadmium	16	32	0	4,17	16	4	0	4,17	0,5	2	4	4,17	133,35	158,33	16,67	110,42	83,33	1,33	19,00	1,65	182,55				
	Chromium	2	32	0	4,17	0,5	1	0	4,17	8	8	0	4,17	41,68	170,83	0,00	63,55	75,00	0,85	13,00	1,41	89,81				
	Mercury	2	32	0	4,17	16	4	0	4,17	8	8	0	4,17	108,33	183,33	0,00	100,00	75,00	1,33	15,00	1,49	149,32				
	Nichel	16	32	0	4,17	4	2	0	4,17	0,5	0,5	4	4,17	83,35	141,68	16,67	81,26	83,33	0,98	19,00	1,65	134,34				
	Lead	16	32	0	4,17	16	4	0	4,17	0,5	2	0	4,17	133,35	158,33	0,00	106,26	75,00	1,42	15,00	1,49	158,66				
	Benzene	1	16	32	3,54	2	1	2	4,94	0,5	2	16	4,02	13,43	69,57	187,38	70,95	99,98	0,71	9,33	1,27	89,86				
	PAHs	0,5	0,5	2	4,59	16	8	2	6,91	0,5	0,5	2	1,00	110,52	55,27	25,00	75,33	100,01	0,75	12,00	1,37	103,44				
	Dioxins	4	4	32	7,37	4	0,5	4	4,12	8	8	8	1,01	54,02	37,55	260,24	101,46	99,96	1,02	14,00	1,45	147,44				
	PCBs	8	8	32	7,37	4	8	8	4,12	8	8	8	1,01	83,48	99,96	276,72	135,91	99,96	1,36	14,00	1,45	197,50	145,91	222,71	146,06	
	HCB	1	32	32	9,90	0,5	0,5	2	1,46	8	8	8	1,14	19,03	325,98	328,89	173,23	100,02	1,73	13,00	1,41	244,81				
C a s e	DDT	4	16	32	6,70	32	1	2	4,77	8	8	8	1,02	187,73	120,15	232,13	181,93	99,97	1,82	12,00	1,37	249,83				
	Atrazine	4	2	32	9,28	32	0,5	2	2,21	8	8	8	1,00	115,98	26,58	309,52	142,02	99,99	1,42	14,00	1,45	206,38				
	NOx									12	12							12,0				12,00				
	SOx									12	70							41,0				41,00				
	CO									605	70							337,5				337,50				
	O3									70	70							70,0				70,00				
	PM10									12	12							12,0				12,00				
	PM2.5									139	12							75,5				75,50				
	C a s e	Arsenic	4	32	32	4,17	8	8	8	4,17	0,5	0,5	2	4,17	50,0	166,7	175,0	110,4	100,0	1,1	19,0	1,65	182,56			
		Cadmium	16	32	0	4,17	8	8	0	4,17	0,5	1	4	4,17	100,0	170,8	16,7	96,9	83,3	1,2	19,0	1,65	160,17			
		Chromium	4	16	0	4,17	8	8	0	4,17	1	0,5	0	4,17	54,2	100,0	0,0	52,1	75,0	0,7	13,0	1,41	73,61			
Mercury		2	32	0	4,17	8	8	0	4,17	0,5	1	0	4,17	41,7	170,8	0,0	63,5	75,0	0,8	15,0	1,49	94,89				
Nichel		4	32	0	4,17	8	8	0	4,17	0,5	0,5	4	4,17	50,0	166,7	16,7	70,8	83,3	0,9	19,0	1,65	117,12				
Lead		16	32	0	4,17	8	8	0	4,17	0,5	1	0	4,17	100,0	170,8	0,0	92,7	75,0	1,2	15,0	1,49	138,44				
Benzene		8	8	8	3,54	8	8	8	4,94	0,5	0,5	1	4,02	67,8	67,8	71,8	68,8	100,0	0,7	9,3	1,27	87,17				
PAHs		0,5	0,5	32	4,59	8	8	8	6,91	0,5	0,5	2	1,00	55,3	55,3	204,2	92,5	100,0	0,9	12,0	1,37	127,01				
Dioxins		4	2	32	7,37	8	8	8	4,12	0,5	1	2	1,01	62,4	48,7	270,7	111,1	100,0	1,1	14,0	1,45	161,38	121,88	186,02	114,30	
PCBs		4	2	32	7,37	8	8	8	4,12	0,5	1	2	1,01	62,4	48,7	270,7	111,1	100,0	1,1	14,0	1,45	161,38				
HCB		8	8	8	9,90	8	8	8	1,46	8	8	8	1,14	100,0	100,0	100,0	100,0	100,0	1,0	13,0	1,41	141,34				
DDT	8	8	8	6,70	8	8	8	4,77	8	8	8	1,02	100,0	100,0	100,0	100,0	100,0	1,0	12,0	1,37	137,27					
Atrazine	8	8	8	9,28	8	8	8	2,21	8	8	8	1,00	100,0	100,0	100,0	100,0	100,0	1,0	14,0	1,45	145,31					
NOx										12	12						12,0				12,0					
SOx										12	12						12,0				12,0					
CO										139	139						139,0				139,0					
O3										12	12						12,0				12,0					
PM10										12	70						41,0				41,0					
PM2.5										605	139						372,0				372,0					

Fig 4.2 Pilot case and law limit case values in ERICA classification diagram.



ERICA gives a score of 146.06 for the Italian limit scenario, corresponding to an "unhealthy for sensitive groups" classification while for the pilot case, where data on pollutants' environmental levels are available, ERICA gives a score of 114.30 corresponding to an "unhealthy for sensitive groups" scenario (Fig. 4.2).

In the Italian legislation, there are no defined limits for a few pollutants in some environmental compartments. Furthermore, the legal limits are derived from many considerations such as toxicology, politics and recent advances in technology and they do not, in some cases, reflect the application of health and ecological risk limits. Instead, the overall ERICA depicts a health scenario focused on the health risk limit for all the environmental compartments.

The high ERICA value using the Italian legal limit reflects the threshold value for risk for the environmental status considered complete assuming the risk limit where the legal restrictions are missing.

4.8 Analysis of the pilot case study

The following substances in air and soils in the Sant' Urbano Northern Italy municipality were detected in concentrations exceeding the legal limits:

- PM10 (places 1 and 2) compared to the value established for the human health risk assessment of PM10 in air (D.M. 60/2002);
- Nickel (place 2) compared to the limit value in environmental air as established from European Directive 2004/107/CE
- Cadmium (place 8) compared to the limit value in environmental air as established from European Directive 2004/107/CE
- Tin (geometric mean of the soils content) compared to limit value established in soil for green area, private use and residential (D.Lgs. 152/2006).

The analysis of the impact on human health defines possible concern related to some pollutants in soil. The maximum contribution to this risk is posed by the dermal contact with the soil. Regarding air there are possible risks due to the cumulative effects of toxic chemicals.

VOC analysis also depicted a possible hazard for chlorinate compounds and terpenes in some places.

Furthermore in all places there are high values of PM 2.5 and for Antimony, Cadmium, Lead and Vanadium in the geometric mean of soil samples.

4.9 Discussion about pilot case

The risk evaluation analysis conducted in our laboratories to define the environmental and toxic risks related to the landfill emissions report gave comparable results with ERICA value. The compounds exceeding the Italian law limit were: PM10, Ni, Cd, Sn in air and soil samples monitored during 3 months in

different areas. The detailed risk analysis showed possible risks for sensitive groups related to the dermal contact within the soil.

Furthermore the values of PM2.5, Sb, Cd, Pb and V show a possible risk for sensitive groups and environment.

ERICA condensed value defines in a unique number the possible risks for sensitive groups and the single values of SRI of each compounds well identify the risks reported in Table 4.5.

CONCLUSIONS

A risk assessment strategy considering the impact of chemicals on the whole ecosystem has been developed, taking into consideration the physico-chemical, toxicological and ecotoxicological properties related to various species and environmental compartments.

The condensed information provided by ERICA makes it easy to classify the health of a territory even following time variations. The approach is general and flexible. Additional information like new alternative chemicals of interest (such as pharmaceuticals) and new toxicological characteristics may be added, on the condition that the added information is complete and available also for the compounds of the minimum scenario.

It is possible to enlarge the ERICA results with new risk assessment methodologies like in vitro assays and/or human biomarkers (e.g. occupational biomarkers).

The condensed information is a relevant opportunity in risk communication and perception for both regulators and population.

ERICA is an easy criterion for the classification of the health status of an investigated site in both spatial and temporal dimensions, and it may be easily modulated in the future by other information provided by different kinds of assays (e.g. ecotoxicological tests on soil organisms, in vitro responses on pulmonary cells) or from epidemiological and socio-economic studies.

This enlarged point of view of the ERICA applicability is valuable to study the effects of mixtures of pollutants, the nature of the interactive effects of compounds on the target organisms and territories.

Another way to use data coming from ecotoxicological assays, biomarkers, cell assays or epidemiological studies in ERICA could be their direct implementation from the very beginning, as toxicological or ecotoxicological parameters as PNECs values, slope factors or reference doses.

In conclusion ERICA considers the site-specific chemical loads and can be further analyzed to define a single chemical change and the inherent toxicity or to group the overall chemical impact and potential effect in a given area.

ERICA is intended as a concise and transparent decision tool for environmental policy because:

- 1) It takes into account how far the healthiness of the investigated site is from the risk threshold, so it is not only an acceptable or not-binary classification;
- 2) It takes into account the overall mixture of pollutants assessing their toxicological and ecotoxicological weights and giving a picture of their impact;
- 3) It can manage cases with few data, thanks to predictive methods;
- 4) It allows an impartial judgment on the health of a territory, communicating in a straightforward manner;
- 5) It allows an overall evaluation of the time trend of environmental impact on a studied location;
- 6) It can be used to compare spatial situations (in different scales) which otherwise can be compared with difficulties;
- 7) ERICA can be a useful tool for the estimation of "toxicity flux" relayed to the international transport of material, such as waste.

These points are very useful for regulators and scientists how aims to measure the type of risk posed by a certain chemical or by a certain mixture of chemicals. The Index is able to prioritize contaminants and to help regulators in making decisions.

Predictive methods give ERICA the ability to perform risk assessment also if the quality of experimental data are not good. Furthermore, ERICA is a flexible system and new predictive methods can be implemented with their new versions and consequently better results.

ERICA is a valid answer to the recently described need of analytical tools for integrated environmental and health impact assessment (Bhatia and Wernham, 2008). In fact, the need to describe the ecosystem as a complete media for humans and environmental life is becoming the first requirement for improving sustainability of a territory.

The establishment of metrics to prioritize contaminants and environmental monitoring are necessary components of any strategy related to sustainability. ERICA provides snapshots of the existing environmental state and possible hazard for the population. Metrics are defined in relation to clearly stated questions such as the condition of water, soil, air and the overall trends related to the ecological processes that sustain the territory. Over time, I think that ERICA will become a good instrument able to contribute greatly to sustainability by providing important scientific information for sound policy and decision making in several sectors of society.

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Appendix 2

Tab. 1. Ecotoxicological risk assessment: scoring system for EQ values from eq. 1

EQ Scoring system	
EQ	Score
< 0.001	0,5
0.001-0.01	1
0.01-0.1	2
0.1-1	4
1	8
1-10	16
> 10	32

Eq. A. General formula to calculate the chronic daily intake (CDI)

$$CDI = C \times \frac{CR_{matrix} \times EF \times ED}{BW \times AT \times CF}$$

where

C = concentration of substance in the matrix [mg kg⁻¹; mg m⁻³; mg l⁻¹];

CR_{matrix} = contact rate with the matrix in which substance x is dispersed;

EF = exposure frequency [days year⁻¹];

ED = duration of exposure [years];

BW = target body weight [kg];

AT = average time of exposure to substance x [years] ;

CF = conversion coefficient year-days = 350.

Eq. B. Chronic daily intake by dermal contact with the soil

$$CDI = C \times \frac{SA \times AF \times ABS \times EF \times ED}{BW \times AT \times CF}$$

where

C = concentration of compound in soil and deposition [mg (kg dry soil)⁻¹]

SA = surface of exposed skin [cm²]

AF = factor of dermal adhesion for the soil [mg (cm² day)⁻¹]

ABS = dermal absorption factor

Eq. C. Chronic daily intake by accidental ingestion of soil

$$CDI = C \times \frac{IR \times FI \times CF_w \times EF \times ED}{BW \times AT \times CF}$$

where

C = concentration of compound x in soil and deposition [mg (kg dry soil)⁻¹]

IR = ingestion rate [mg day⁻¹]

FI = ingested fraction of soil

CF_w = conversion factor mg/kg = 1E-6

Eq. D. Chronic daily intake by Inhalation of air

$$CDI = C \times \frac{B \times EF_g \times EF \times ED}{BW \times AT \times CF}$$

Where

C = concentration of compound in air and particulate phase [mgm⁻³]

B = outdoor inhalation [m³ hour⁻¹]

EF_g = daily frequency outdoor exposure [hours day⁻¹]

Eq. E. Chronic daily intake by water ingestion

$$CDI = C \times \frac{IR \times EF \times ED}{BW \times AT \times CF}$$

where

C = concentration of compound in the water [mg/L]

IR = water ingestion rate [L/day]

Tab. 2. Human Risk Assessment: scoring system for HQ values from eq. 6

HQ Scoring system	
HQ	Score
< 0.001	0,5
0.001-0.01	1
0.01-0.1	2
0.1-1	4
1	8
1-10	16
> 10	32

Tab. 3. Cancer Risk Assessment: scoring system for HQ values from eq. 7.

CR Scoring system	
CR	Score
non cancerogenic	0
< 10^{-8}	1
10^{-8} - 10^{-6}	2
10^{-6} - 10^{-5}	4
10^{-5}	8
10^{-5} - 10^{-4}	16
> 10^{-4}	32

Tab. 4. Scoring system for water solubility (eq. 11). Range of solubility from European Pharmacopeia

Water Solubility (W) scoring criteria	
Value	Score
< 10^2 mg/L	1
10^2 - 10^3 mg/L	2
10^3 - 10^4 mg/L	3
10^4 - $3.3 \cdot 10^4$ mg/L	4
$3.3 \cdot 10^4$ - 10^5 mg/L	5
10^5 - 10^6 mg/L	6
> 10^6 mg/L	7

Tab. 5. Scoring system for soil mobility (eq. 11). Range from Wilson et al., 1996.

Soil mobility (M) scoring system	
Value	Score
>5000	1
5000-2000	1,5
2000-500	2
500-150	3
150-50	4
< 50	5

Tab. 6. Scoring system for the volatility of compounds (eq. 11). Range from Duffus et al., 2006.

Volatility (V) scoring criteria	
Value	Score
< 25 mm Hg	1
25-78 mm Hg	2
> 78 mm Hg	3

Tab. 7. Scoring system for the bioconcentration properties (eq. 11). Value range from Snyder et al., 2000.

Bioaccumulation Properties (BCF/BAF)	
Value	Score
< 2	1
2-3	2
3-4	3
4-5	4
> 5	5

Tab. 8. Scoring system for the persistence properties (eq. 11). Range from Snyder et al., 2000.

Persistence (P) scoring criteria	
Value	Score
hours	0
hours - day	1
days	2
days - weeks	3
weeks	4
weeks - months	5
months	6
recalcitrant	7

Tab. 9. Scoring system for the Air Quality Index classification of macropollutants (eq. 17-18). AQI range from USEPA, 2003 and USEPA, 2009.

AQI classification and scoring system		
AQI	Classification	Score
1 - 50	Good	12,5
51 - 100	Moderate	69,7
101 - 150	Unhealthy for sensitive groups	139,3
151 - 200	Unhealthy	151,3
201 - 300	Very Unhealthy	302,6
301 - 500	Hazardous	605,3

Tab 10: industrial plants: Italian law limit values for air

Pollutants	Unit	Law	Mediation period	Law limits		
				Human health limit value	Environmental health limit value	Max number of exceeds for years
SOx (as SO2)	ug/m3	DM 60/2002	1h	350		24
SOx (as SO2)	ug/m3	DM 60/2002	24h	125		3
SOx (as SO2)	ug/m3	DM 60/2002	Year – winter (1 october - 31 march)		20	3
Nox (as NO2)	ug/m3	DM 60/2002	1h	200		24
Nox (as NO2)	ug/m3	DM 60/2002	Year	20	30	
PM10	ug/m3	DM 60/2002	24h	50		35
PM10	ug/m3	DM 60/2002	Year	40		
PM10 from 1 January 2010	ug/m3	DM 60/2002	24h	50		7
PM10 from 1 January 2010	ug/m3	DM 60/2002	Year	20		
CO	mg/m3	DM 60/2002	Max daily mean on 8h	10		
Benzene	ug/m3	DM 60/2002	Year	5		
Pb	ug/m3	DM 60/2002	Year	0.5		
As	ng/m3	Dir 2004/107/CE		6		
Cd	ng/m3	Dir 2004/107/CE		5		
Ni	ng/m3	Dir 2004/107/CE		20		
Total hydrocarbons, in the periods where limits O3 exceed	ug/m3	DPCM 28/3/1983	Mean of 3h in a period of 24h	200		
Ozone	ug/m3	DM 16/5/1996	8h	110		
Ozone	ug/m3	DM 16/5/1996	24h	65		
PAH (as Benzo(a)pirene)	ng/m3	DM 25/11/1994	Year	1		

Tab 11: industrial plants: Italian law limit values for superficial water

Industrial Plants		
Superficial water		
Limit values		
Pollutants	Unit	Law limit
	[u.d.m.]	D.Lgs. 152/2006
As	µg/l	10.0
Cd+ compounds	µg/l	1.0
Total Cr	µg/l	50.0
Hg +compounds	µg/l	1.0
Ni + compounds	µg/l	20.0
Pb + compounds	µg/l	10.0
PAH	µg/l	0.2
Benzene	µg/l	1.0
TriChloroBenz ene	µg/l	0.4
Biocides	µg/l	1.0
Aldrin	µg/l	0.1
DiEldrin	µg/l	0.1
Endrin	µg/l	0.1
Isodrin	µg/l	0.1
Dichlorodiphen ilchloroethane	µg/l	0.1
EndoSulfan	µg/l	0.1
Alfa EndoSulfan	µg/l	0.1
Esachlorocycl oesane	µg/l	0.1
Lindane	µg/l	0.1
Esachlorobenz ene	µg/l	0.1
Diuron	µg/l	0.1
Isoproturon	µg/l	0.1
Atrazine	µg/l	0.1
Simazine	µg/l	0.1
Clorfenvinos	µg/l	0.1
Clorpyrifos	µg/l	0.1
Alachlor	µg/l	0.1
Trifluralin	µg/l	0.1
Pentachloroph enol	µg/l	0.4
1,2- dichloroethane	µg/l	10.0
chloroethilen	µg/l	0.5
Dichlorometha ne	µg/l	20.0
Esachlorobuta diene	µg/l	0.1
Trichlorometha ne	µg/l	12.0
Trichloroetilene	µg/l	10.0
Tetrachloroethi len	µg/l	10.0

Tab 12: industrial plants: Italian law limit values for groundwater – drinking use

Industrial Plants					
Groundwater					
Groundwater – drinking use					
Pollutants	Unit	Law limits D.Lgs. 152/2006 – Classification			
		A1	A2	A3	A3
	[u.d.m.]				I
Colour	mg/l scala pt	10	100	50	200
	mg/l MES	25	-	-	-
Temperature	°C	22	25	22	25
Conductivity	uS/cm a 20°C	1000		1000	
Odor	dilution factor a 20°C	3	-	20	-
Nitrate	mg/l NO3	25	50	-	50
Fluorurate	mg/l F	0,7/1	-	0,7/1,7	-
Total organic	mg/l Cl	-	-	-	-
Fe	mg/l	0.1	2	1	-
Mn	mg/l	0.05	-	1	-
Cu	mg/l	0,02	-	1	-
Zn	mg/l	0,5	5	1	5
B	mg/l	1	-	1	-
Be	mg/l	-	-	-	-
Co	mg/l	-	-	-	-
Ni	mg/l	-	-	-	-
V	mg/l	-	-	-	-
As	mg/l	0,01	0,05	0,05	0,1
Cd	mg/l	0,001	0,005	0,001	0,005
Total Cr	mg/l	-	0,05	-	0,05
Pb + compounds	mg/l	-	0,05	-	0,05
Se	mg/l	-	0,01	-	0,01
Hg + compounds	mg/l	0,0005	0,001	0,0005	0,001
B	mg/l	-	1	-	1
CN	mg/l	-	0,05	-	0,05
Sulfate	mg/l	150	250	150	250
Chlorurate	mg/l	200	-	200	-
Phosphates	mg/l P2O5	0,4	-	0,7	-
Phenols	mg/l C6H5OH	-	0,005	0,01	0,1
Idrocarbons solved or emulsified	mg/l	-	0,2	0,5	1
PAH	mg/l	-	0,0002	-	0,001
Total biocides	mg/l	-	0,0025	-	0,005
COD	mg/l O2	-	-	30	-
O2 saturation	%O2	>70	-	>30	-
BOD5	mg/l O2	<3	-	<7	-
N Kjeldahl (not	mg/l NO3	1	-	3	-
NH3	mg/l NH4	0,05	1,5	2	4

Tab 13: industrial plants: Italian law limit values for soil – subsurface soil

Pollutants	Unit	Law limit	Law limit
		Use: green and residential	Use: industrial and commercial
		D.Lgs. 152/200	D.Lgs. 152/2006
Sb	mg/Kg dry soil	10	30
As	mg/Kg dry soil	20	50
Benzene	mg/Kg dry soil	2	10
Cd	mg/Kg dry soil	2	15
Co	mg/Kg dry soil	20	250
Total Cr	mg/Kg dry soil	150	800
Cr VI	mg/Kg dry soil	2	15
Hg	mg/Kg dry soil	1	5
Nitrobenzene	mg/Kg dry soil	120	500
Pb	mg/Kg dry soil	100	1000
Cu	mg/Kg dry soil	120	600
Se	mg/Kg dry soil	3	15
Sn	mg/Kg dry soil	1	350
Tl	mg/Kg dry soil	1	10
V	mg/Kg dry soil	90	250
Zn	mg/Kg dry soil	150	1500
CN	mg/Kg dry soil	1	100
Florurate	mg/Kg dry soil	100	2000
Benzene	mg/Kg dry soil	0,1	2
Ethylbenzene	mg/Kg dry soil	0,5	50
Styrene	mg/Kg dry soil	0,5	50
Toluene	mg/Kg dry soil	0,5	50
Xylene	mg/Kg dry soil	0,5	50
Ethylbenzene+ Styrene+ Toluene+ Xylene	mg/Kg dry soil	1	100
Benzo(a)antracene	mg/Kg dry soil	0,5	10
Benzo(a)pyrene	mg/Kg dry soil	0,1	10
Benzo(b)fluorantene	mg/Kg dry soil	0,5	10
Benzo(k)fluorantene	mg/Kg dry soil	0,5	10
Benzo(g, h, i)perylene	mg/Kg dry soil	0,1	10
Crysene	mg/Kg dry soil	5	50
Dibenzo(a, e)pyrene	mg/Kg dry soil	0,1	10
Dibenzo(a, l)pyrene	mg/Kg dry soil	0,1	10
Dibenzo(a, i)pyrene	mg/Kg dry soil	0,1	10
Dibenzo(a, h)pyrene	mg/Kg dry soil	0,1	10
Dibenz(a, h)antracene	mg/Kg dry soil	0,1	10
Indenopyrene	mg/Kg dry soil	0,1	5
Pyrene	mg/Kg dry soil	5	50
ΣPAH	mg/Kg dry soil	10	100
Chloromethane	mg/Kg dry soil	0,1	5
Dichloromethane	mg/Kg dry soil	0,1	5
Trichloromethane	mg/Kg dry soil	0,1	5
Vinyl chloride	mg/Kg dry soil	0,01	0,1
1,2-Dichloroethane	mg/Kg dry soil	0,2	5
1, 1-Dichloroethilen	mg/Kg dry soil	0,1	1
Trichloroethilen	mg/Kg dry soil	1	10
Tetrachloroethilen	mg/Kg dry soil	0,5	20
1,1-Dichloroethane	mg/Kg dry soil	0,5	30
1,2-Dichloroethylene	mg/Kg dry soil	0,3	15
1, 1, 1-Trichloroethane	mg/Kg dry soil	0,5	50
1,2-Dichloropropane	mg/Kg dry soil	0,3	5
1, 1, 2-Trichloroethane	mg/Kg dry soil	0,5	15
1,2,3-Trichloropropane	mg/Kg dry soil	1	10
1, 1, 2, 2-Tetracloroethane	mg/Kg dry soil	0,5	10
Bromoform	mg/Kg dry soil	0,5	10
Ethylene dibromide	mg/Kg dry soil	0,01	0,1
Chlorodibromomethane	mg/Kg dry soil	0,5	10
Bromodichloromethane	mg/Kg dry soil	0,5	10
Nitrobenzene	mg/Kg dry soil	0,5	30
1,2-Dinitrobenzene	mg/Kg dry soil	0,1	25
1,3-Dinitrobenzene	mg/Kg dry soil	0,1	25
chloronitrobenzene	mg/Kg dry soil	0,1	10
Monochlorobenzene	mg/Kg dry soil	0,5	50
1,2,4-trichlorobenzene	mg/Kg dry soil	1	50
1,2,4,5-Tetrachlorobenzene	mg/Kg dry soil	1	25
Pentachlorobenzene	mg/Kg dry soil	0,1	50
Hexachlorobenzene	mg/Kg dry soil	0,05	5